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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

JAZZ PHARMACEUTICALS IRELAND, LIMITED, Plaintiffs, v. LUPIN INC. and LUPIN PHARMACEUTICALS, INC., Defendants.	Honorable Stanley R. Chesler, U.S.D.J. Civil Action No. 21-14271 (SRC)(JSA) Civil Action No. 22-2773 (SRC)(JSA) Civil Action No. 23-329 (SRC)(JSA) (Consolidated) **CORRECTED - DECLARATION OF JAMES S. RICHTER SUBMITTED IN SUPPORT OF DEFENDANTS' OPENING CLAIM CONSTRUCTION BRIEF**

JAMES S. RICHTER, of full age, hereby declares as follows:

1. I am an attorney at law of the State of New Jersey and Of Counsel with Midlige Richter, LLC, who along with McGuireWoods LLP, are attorneys for Defendants, Lupin Inc. and Lupin Pharmaceuticals, Inc. ("Lupin") in the above-captioned matter.

2. This Declaration is submitted in support of Defendants' Opening Claim Construction Brief.

3. Attached hereto as Exhibit 1 is a true copy of U.S. Patent No. 11,426,373.

4. Attached hereto as Exhibit 2 is a true copy of "Guidance for Industry: Food-Effect Bioavailability and Fed Bioequivalent Studies," FDA, December 2022.

5. Attached hereto as Exhibit 3 is a true copy of the Notice of Allowability, Application No. 17/131,418, Mail Date 04/20/2022.

6. Attached hereto as Exhibit 4 is a true copy of the Declaration of Panayiotis P. Constantinides, Ph.D, including Exhibits 1-5 thereto.

7. Attached hereto as Exhibit 5 is excerpts from Jazz's Responses to Defendants' Invalidity Contentions Regarding U.S. Patent Nos. 11,426,373 and 11,552,102. (Filed Under Seal).

I hereby declare under the penalty of perjury that the foregoing statements made by me are true and correct.

s/ James S. Richter
James S. Richter
jrichter@winston.com

Dated: October 5, 2023

EXHIBIT 1

US011426373B2

(12) **United States Patent**
Allphin et al.

(10) **Patent No.: US 11,426,373 B2**
(45) **Date of Patent: Aug. 30, 2022**

(54) **GAMMA-HYDROXYBUTYRATE COMPOSITIONS AND THEIR USE FOR THE TREATMENT OF DISORDERS**

(71) Applicant: **Jazz Pharmaceuticals Ireland Limited**, Dublin (IE)

(72) Inventors: **Clark P. Allphin**, Seattle, WA (US); **Gunjan Junnarkar**, Palo Alto, CA (US); **Roman Skowronski**, Palo Alto, CA (US); **Cuiping Chen**, Palo Alto, CA (US); **Katayoun Zomorodi**, San Jose, CA (US); **Mark Eller**, Redwood City, CA (US)

(73) Assignee: **Jazz Pharmaceuticals Ireland Limited**, Dublin (IE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **17/131,418**

(22) Filed: **Dec. 22, 2020**

(65) **Prior Publication Data**

US 2021/0121423 A1 Apr. 29, 2021

Related U.S. Application Data

(63) Continuation of application No. 16/575,213, filed on Sep. 18, 2019, now abandoned, which is a continuation of application No. 15/709,262, filed on Sep. 19, 2017, now abandoned.

(60) Provisional application No. 62/473,232, filed on Mar. 17, 2017.

(51) **Int. Cl.**
A61K 31/19 (2006.01)
A61P 25/20 (2006.01)

(52) **U.S. Cl.**
CPC ***A61K 31/19*** (2013.01); *A61K 2300/00* (2013.01)

(58) **Field of Classification Search**
CPC A61K 31/19; A61P 25/20
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,051,619	A	8/1962	Laborit
3,419,588	A	12/1968	De Man
4,221,778	A	9/1980	Raghunathan
4,374,441	A	2/1983	Carter et al.
4,393,236	A	7/1983	Klosa
4,510,128	A	4/1985	Khanna
4,524,217	A	6/1985	Davenport et al.
4,687,662	A	8/1987	Schobel
4,738,985	A	4/1988	Kluger et al.
4,916,161	A	4/1990	Patell
4,939,949	A	7/1990	Langenberg
4,983,632	A	1/1991	Gessa et al.
5,294,430	A	3/1994	Borch et al.
5,380,937	A	1/1995	Koehler et al.
5,415,870	A	5/1995	Gergely et al.
5,594,030	A	1/1997	Conte et al.
5,753,708	A	5/1998	Koehler et al.
5,758,095	A	5/1998	Albaum et al.
5,833,599	A	11/1998	Schrier et al.
5,840,331	A	11/1998	Van Cauter et al.
5,845,255	A	12/1998	Mayuad
5,955,106	A	9/1999	Moeckel et al.
5,990,162	A	11/1999	Sharf
6,014,631	A	1/2000	Teagarden et al.
6,022,562	A	2/2000	Autant et al.
6,067,524	A	5/2000	Byerly et al.
6,112,182	A	8/2000	Akers et al.
6,317,719	B1	11/2001	Schrier et al.
6,322,819	B1	11/2001	Burnside et al.
6,356,873	B1	3/2002	Teagarden et al.
6,384,020	B1	5/2002	Flanner et al.
6,436,998	B1	8/2002	Cacciaglia et al.
6,472,431	B2	10/2002	Cook et al.
6,472,432	B1	10/2002	Perricone
6,495,598	B1	12/2002	Yoneda et al.
6,565,872	B2	5/2003	Wu et al.
6,780,889	B2	8/2004	Cook et al.
7,015,200	B2	3/2006	Mamelak et al.
7,072,840	B1	7/2006	Mayuad
7,262,219	B2	8/2007	Cook et al.
7,568,822	B2	8/2009	Ibrahim
7,668,730	B2	2/2010	Reardan et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2 112663 C	4/2002
CA	2 510 289 A1	7/2004

(Continued)

OTHER PUBLICATIONS

"Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies." U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Dec. 2002. (Year: 2002).*

Borgen et al. (The Influence of Gender and Food on the Pharmacokinetics of Sodium Oxybate Oral Solution in Healthy Subjects. Journal of Clinical Pharmacology, 2003;43:59-65). (Year: 2003).*

"HIB-IMUNE," Physicians Desk Reference (41st ed.), (1987), 1095-1096.

"HibVAX," Physicians Desk Reference (41st ed.), (1987), 870.

"Phospholine Iodide," Physicians Desk Reference (50th ed.), (1996), 2784.

"Taxotere," Physicians Desk Reference (51st ed.), (1997), 2204-2207.

(Continued)

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(74) *Attorney, Agent, or Firm* — Cooley LLP

(57) **ABSTRACT**

Provided herein are pharmaceutical compositions and formulations comprising mixed salts of gamma-hydroxybutyrate (GHB). Also provided herein are methods of making the pharmaceutical compositions and formulations, and methods of their use for the treatment of sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

11 Claims, 6 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,765,106 B2 7/2010 Reardan et al.
7,765,107 B2 7/2010 Reardan et al.
7,797,171 B2 9/2010 Reardan et al.
7,851,506 B2 12/2010 Cook et al.
7,895,059 B2 2/2011 Reardan et al.
8,101,209 B2 1/2012 Legrand et al.
8,193,211 B2 6/2012 Liang et al.
8,202,537 B2 6/2012 Mehta et al.
8,263,125 B2 9/2012 Vaya et al.
8,263,650 B2 9/2012 Cook et al.
8,324,275 B2 12/2012 Cook et al.
8,457,988 B1 6/2013 Reardan et al.
8,461,197 B2 6/2013 Tung
8,461,203 B2 6/2013 Cook et al.
8,529,954 B2 9/2013 Lebon et al.
8,589,182 B1 11/2013 Reardan et al.
8,591,922 B1 11/2013 Allphin et al.
8,598,191 B2 12/2013 Liang et al.
8,680,228 B2 3/2014 Guo et al.
8,731,963 B1 5/2014 Reardan et al.
8,759,394 B2 6/2014 Tung et al.
8,771,735 B2 7/2014 Rourke et al.
8,772,306 B1 7/2014 Eller
8,778,301 B2 7/2014 Mamelak et al.
8,778,398 B2 7/2014 Rourke et al.
8,859,619 B2 10/2014 Cook et al.
8,901,173 B2 12/2014 Allphin et al.
8,952,029 B2 2/2015 Eller
8,952,062 B2 2/2015 Cook et al.
9,023,400 B2 5/2015 Guimberteau et al.
9,050,302 B2 9/2015 Eller
9,132,107 B2 9/2015 Allphin et al.
9,486,426 B2 11/2016 Eller
9,539,330 B2 1/2017 Cook et al.
9,555,017 B2 1/2017 Allphin et al.
9,770,514 B2 9/2017 Ghebre-Sellassie
9,795,567 B2 10/2017 Rourke et al.
9,801,852 B2 10/2017 Allphin
10,195,168 B2 2/2019 Allphin et al.
10,213,400 B2 2/2019 Eller
10,272,062 B2 4/2019 Mégret et al.
10,398,662 B1 9/2019 Allphin et al.
10,736,866 B2 8/2020 Mégret et al.
10,758,488 B2 9/2020 Allphin et al.
10,813,885 B1 10/2020 Allphin et al.
10,925,844 B2 2/2021 Grassot et al.
10,952,986 B2 3/2021 Megret et al.
10,959,956 B2 3/2021 Allphin et al.
10,966,931 B2 4/2021 Allphin et al.
10,973,795 B2 4/2021 Megret et al.
10,987,310 B2 4/2021 Allphin et al.
11,077,079 B1 8/2021 Allphin et al.
11,090,269 B1 8/2021 Allphin et al.
2003/0180249 A1 9/2003 Khanna et al.
2004/0092455 A1 5/2004 Mamelak et al.
2005/0031688 A1 2/2005 Ayala
2005/0037077 A1 2/2005 Legrand et al.
2005/0113366 A1 5/2005 Bourguignon et al.
2005/0142192 A1 6/2005 Benjamin et al.
2006/0018933 A1 1/2006 Vaya et al.
2006/0024365 A1 2/2006 Vaya et al.
2006/0069040 A1 3/2006 Mamelak
2006/0210630 A1 9/2006 Liang et al.
2006/0228410 A1 10/2006 Dumont et al.
2007/0270491 A1 11/2007 Cook et al.
2008/0003267 A1 1/2008 Spencer et al.
2008/0069871 A1 3/2008 Vaughn et al.
2008/0085304 A1 4/2008 Baichwal et al.
2008/0118571 A1 5/2008 Lee et al.
2008/0226564 A1 9/2008 Weers et al.
2008/0292700 A1 11/2008 Nghiem et al.
2008/0293698 A1 11/2008 Johnson
2009/0137565 A1 5/2009 Frucht
2009/0155357 A1 6/2009 Muhuri
2009/0317355 A1 12/2009 Roth et al.
2010/0112056 A1 5/2010 Rourke et al.
2010/0266701 A1 10/2010 Guimberteau et al.
2011/0034727 A1 2/2011 Luchi et al.
2011/0039929 A1 2/2011 Cook et al.
2011/0091537 A1 4/2011 Castan et al.
2011/0111027 A1 5/2011 Rourke et al.
2011/0213004 A1 9/2011 Kim et al.
2012/0020833 A1 1/2012 Cook et al.
2012/0076865 A1 3/2012 Allphin et al.
2012/0148672 A1 6/2012 Mehta et al.
2012/0202879 A1 8/2012 Cook et al.
2012/0202880 A1 8/2012 Cook et al.
2013/0230587 A1 9/2013 Pilgaonkar et al.
2013/0273159 A1 10/2013 Howard et al.
2014/0004202 A1 1/2014 Suplie et al.
2014/0037745 A1 2/2014 Liang et al.
2014/0072624 A1 3/2014 Jung et al.
2014/0093578 A1 4/2014 Mehta et al.
2014/0127306 A1 5/2014 Mehta et al.
2014/0141090 A1 5/2014 Wilson
2014/0171506 A1 6/2014 Allphin et al.
2014/0271896 A1 9/2014 Abu Shmeis et al.
2014/0348917 A1 11/2014 Rourke et al.
2015/0005334 A1 1/2015 Shah et al.
2015/0073052 A1 3/2015 Cook et al.
2015/0328168 A1 11/2015 Daviaud-Venet et al.
2016/0068463 A1 3/2016 Peoples et al.
2016/0228379 A1 8/2016 Kumar et al.
2016/0271070 A1 9/2016 Singh et al.
2016/0338966 A1 11/2016 Guimberteau et al.
2016/0346200 A1 12/2016 Sommer et al.
2016/0346216 A1 12/2016 Chen
2017/0119627 A1 5/2017 Bhargava et al.
2017/0340519 A9 11/2017 Bhargava et al.
2018/0008539 A1 1/2018 Singh et al.
2018/0021284 A1 1/2018 Mégret et al.
2018/0042855 A1 2/2018 Rourke et al.
2018/0263936 A1 9/2018 Allphin et al.
2018/0318222 A1 11/2018 Allphin et al.
2019/0183806 A1 6/2019 Guillard
2019/0183836 A1 6/2019 Mégret et al.
2019/0269640 A1 9/2019 Mégret et al.
2019/0269641 A1 9/2019 Mégret et al.
2019/0274990 A1 9/2019 Mégret et al.
2019/0282532 A1 9/2019 Mégret et al.
2020/0113840 A1 4/2020 Allphin et al.
2020/0197347 A1 6/2020 Mégret et al.
2020/0276142 A1 9/2020 Grassot et al.
2020/0330393 A1 10/2020 Walsh et al.
2020/0360293 A1 11/2020 Guillard
2020/0360319 A1 11/2020 Grassot et al.
2020/0368187 A1 11/2020 Grassot et al.
2021/0186907 A1 6/2021 Skobieranda

FOREIGN PATENT DOCUMENTS

CN 102905688 A 1/2013
CN 102958930 A 3/2013
CN 103209966 A 7/2013
CN 103209967 A 7/2013
EP 0203768 A2 12/1986
EP 0235408 A1 9/1987
EP 0344704 A1 6/1989
EP 0635265 A1 7/1994
EP 0616804 A1 9/1994
EP 0635265 A1 1/1995
EP 0709087 B1 12/1999
EP 1140061 A2 10/2001
EP 1140061 B1 10/2001
EP 1316309 A1 6/2003
EP 2760911 B1 11/2017
EP 1434572 B1 12/2017
GB 922029 3/1963
GB 2295390 A 5/1996
JP 57-042651 3/1982
JP 62-12715 A 1/1987
JP 04-049212 2/1992
JP 05-508422 11/1993
JP H06-508839 A 10/1994

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

JP	7-53365	A	2/1995
JP	H8-511257	A	11/1996
JP	09-104620	A	4/1997
JP	H10-505604	A	6/1998
JP	2001-513552	A	9/2001
JP	2002533388	A	10/2002
JP	2004-514732	A	5/2004
JP	2007-521231	A	8/2007
JP	2008-512386	A	4/2008
JP	2008-519847	A	6/2008
JP	2008-528571	A	7/2008
JP	2009-532331	A	9/2009
JP	2011-500865	A	1/2011
JP	2012507532	A	3/2012
RU	2210360	C1	8/2003
WO	WO 1994/028880	A1	12/1994
WO	WO 9640105	A1	12/1996
WO	WO 1999/009972	A1	3/1999
WO	WO 0038672	A2	7/2000
WO	WO 2002/045684	A2	6/2002
WO	WO 2005/016318	A1	2/2005
WO	WO 2005/099671	A2	10/2005
WO	WO 2006/029155	A2	3/2006
WO	WO 2006/053186	A2	5/2006
WO	WO 2006/080029	A1	8/2006
WO	WO 2007/053698	A2	5/2007
WO	WO 2007/103200	A2	9/2007
WO	WO 2008/086804	A2	7/2008
WO	WO 2009/056550	A2	5/2009
WO	WO 2010/055260	A1	5/2010
WO	WO 2010053691	A1	5/2010
WO	WO 2011/119839	A1	9/2011
WO	WO 2011/127252	A2	10/2011
WO	WO 2011/135461	A2	11/2011
WO	WO 2011/140310	A2	11/2011
WO	WO 2011139271	A1	11/2011
WO	WO 2012/028688	A1	3/2012
WO	WO 2012/107652	A1	8/2012
WO	WO 2014/078014	A2	5/2014
WO	WO 2014/093791	A1 *	6/2014
WO	WO 2015/120006	A1	8/2015
WO	WO 2015/120110	A2	8/2015
WO	WO 2015/166473	A1	11/2015
WO	WO 2016/087952	A1	6/2016
WO	WO 2016/178132	A1	10/2016
WO	WO 2017/147375	A1	8/2017
WO	WO 2017/182851	A1	10/2017
WO	WO 2018/015563	A1	1/2018
WO	WO 2019/123269	A1	6/2019
WO	WO 2020/178695	A1	9/2020

OTHER PUBLICATIONS

21 C.F.R. 184, Food and Drug Administration, HHS, (1998), pp. 441-535.

Activase, Physicians Desk Reference (50th ed.), (1996), pp. 312,1058-1061.

Akifuddin et al. "Preparation, characterization and in-vitro evaluation of microcapsules for controlled release of Diltiazem hydrochloride by Ionotropic gelation technique." Journal of Applied Pharmaceutical Science (2013); 3.4: 35-42.

Alshaikh et al., "Sodium Oxybate for Narcolepsy with Cataplexy: Systematic Review and Meta-Analysis," Journal of Clinical Sleep Medicine, 2012, vol. 8, No. 4, 451-458.

Anand et al. "Ion-exchange resins: carrying drug delivery forward." Drug Discovery Today (2001); 6.17: 905-914.

Baldrick, P., "Pharmaceutical Excipient Development: The Need for Preclinical Guidance," Regul. Toxicol. Pharmacol. Oct. 2000 32(2):210-218.

Bodmeier, R., "Tableting of coated pellets," European Journal of Pharmaceutics and Biopharmaceutics, (1997) 43(1), 1-8.

Borgen et al., "The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects." J Clin Pharmacol. (2003); 43(1): 59-65.

Borgen, L., et al. "Xyrem® (sodium oxybate): A Study of Dose Proportionality in Healthy Human Subjects." J. Clin. Pharmacol. (2000); 40:1053.

Broughton, et al. "Effects of Nocturnal Gamma-Hydroxybutyrate on Spell/Waking Patterns in Narcolepsy-Cataplexy." Can J. Neural Sci (1980); 7 (1): 23-31.

Broughton, et al. "Gamma-Hydroxy-Butyrate in the Treatment of Narcolepsy: a Preliminary Report." (1976) Narcolepsy, Ny, N.Y., Spectrum Publications, Inc. 659-668.

Caballero et al. "Characterization of alginate beads loaded with ibuprofen lysine salt and optimization of the preparation method." International Journal of Pharmaceutics (2014); 460.1: 181-188.

Chern Abstract ES302338, SciFinder®, (1964), 1 pg.

Chemical Abstracts: Seventh Collective Index, vols. 56-65, (1962-1966), 4 pgs.

Davis et al. "Active chloride secretion in the normal human jejunum." J Clin Invest. (1980); 66(6): 1326-1333.

Frucht, et al. "A pilot Tolerability and Efficacy Trial of Sodium Oxybate in Ethanol-Responsive Movement Disorders." Movement Disorders (2005); 20 (10): 1330-1337.

Gallimberti et al., "Clinical efficacy of gamma-hydroxybutyric acid in treatment of opiate withdrawal," EurArch Psychiatry Clin Neurosci. 1994;244(3):113-114.

Gallimberti et al., "Gamma-Hydroxybutyric Acid for Treatment of Opiate Withdrawal Syndrome," Neuropsychopharmacology, 1993, vol. 9, No. 1, pp. 77-81.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2019/062237, dated Mar. 31, 2020, 11 pages.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2020/066561, dated Apr. 13, 2021, 12 pages.

Jazz Pharmaceuticals, "Jazz Pharmaceuticals Announces Positive Top-line Results from Phase 3 Study of JZP-258 in Adult Narcolepsy Patients with Cataplexy and Excessive Daytime Sleepiness," Mar. 26, 2019, 2 pages, retrieved from https://investor.jazzpharma.com/node/16206/pdf.

Keating, GM, "Sodium Oxybate: A Review of Its Use in Alcohol Withdrawal Syndrome and in the Maintenance of Abstinence in Alcohol Dependence," Clinical Drug Investigation (2014) 34, 63-80.

Khediri et al., "Efficacy of Diosmectite (Smecta)® in the Treatment of Acute Watery Diarrhea in Adults: A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study," Hindawi Publishing Corporation, Gastroenterology Research and Practice, 2011, vol. 2011, Article ID 783196, 8 pages.

Lapierre et al., "The Effect of Gamma-Hydroxybutyrate: A Double-Blind Study of Normal Subjects," Sleep Research (1988); 17:99, 1988, 6 pages. (Abstract Only).

Lubrano, et al. "Fibromyalgia in Patients with Irritable Bowel Syndrome. An Association with the Severity of the Intestinal Disorder." Int J Colorectal Dis. (2001); 16 (4): 211-215.

Luhn, O., "Using Excipients in Powder Formulations," Pharmaceutical Technology Europe, Jan. 7, 2011, vol. 23, Issue 1, 6 pages, retrieved from https://www.pharmtech.com/view/using-excipients-powder-formulations.

Mahore et al. "Ion exchange resins: pharmaceutical applications and recent advancement." Int J Pharm Sci Rev Res (2010); 1.2: 8-13.

Mamelak, M., et al., "Treatment of Narcolepsy and Sleep Apnea with Gammahydroxybutyrate: A clinical and polysomnographic case study." Sleep (1981); 4 (1): 105-111.

Mamelak, M., et al., "Treatment of Narcolepsy with y-hydroxybutyrate. A review of Clinical and Sleep Laboratory Findings." Sleep (1986); 9 (1): 285-290.

Medicines for Children, "Oral Rehydration Salts," Leaflet information published Jul. 25, 2013, by Neonatal and Paediatric Pharmacists Group (NPPG), 6 pages, retrieved from https://www.medicinesforchildren.org.uk/oral-rehyd ration-salts.

Moldofsky et al. "A Chronobiologic Theory of Fibromyalgia." J. Muscoloskel. Pain, 1, 49 (1993).

Moldofsky, et al. "Musculoskeletal Symptoms and Non-REM Sleep Disturbance in Patients with 'Fibrositis Syndrome' and Healthy Subjects." Psychosom. Med. (1975); 37 (4): 341-351.

(56) **References Cited**

OTHER PUBLICATIONS

Morrison, Robert Thornton, et al., Organic Chemistry, 3rd Edition, (1973), pp. 672-677.
Ohta et al. "Development of a simple method for the preparation of a silica gel based controlled delivery system with a high drug content." European Journal of Pharmaceutical Sciences (2005); 26.1: 87-96.
Outlaw, et al. "Dyspepsia and its Overlap with Irritable Bowel Syndrome." Curr Gastroenterol Rep. (2006); 8 (4): 266-272.
Parmar et al., "Clinical Characteristics of Cataplectic Attacks in Type 1 Narcolepsy," Current Neurology and Neuroscience Reports (2020) 20:38, 9 pages.
Patil et al. "A review on ionotropic gelation method: novel approach for controlled gastroretentive gelispheres." International Journal of Pharmacy and Pharmaceutical Sciences (2012); 4.4: 27-32.
Puguan et al. "Diffusion characteristics of different molecular weight solutes in Ca-alginate gel beads." Colloids and Surfaces A: Physicochemical and Engineering Aspects (2015); 469:158-165.
Remington. The Science and Practice of Pharmacy. 20th Edition, Gennaro, Ed,. Lippincott Williams & Wilkins (2000). (See e.g. p. 861).
Remington. The Science and Practice of Pharmacy. 20th Edition, Gennaro, Ed,. Lippincott Williams & Wilkins. Chapter 45 (Oral Solid Dosage Forms) (2000) pp. 889-928.
Rohm and Haas. "Duolite AP143/1083 Pharmaceutical Grade Anion Exchange Resin." Feb. 2006, 4 pages.
Roxane Laboratories, Inc.'s Answer and Affirmative Defenses to Plaintiff's Complaint, (Jan. 4, 2013), 8 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Dec. 29, 2010), 21 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Jun. 1, 2011), 12 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Mar. 9, 2011), 13 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Nov. 9, 2012), 18 pages.
Roxane Laboratories, Inc.'s Initial Invalidity and Noninfringement Contentions Pursuant to Local Patent Rule 3.6, (Apr. 14, 2011), 317 pages.
Rubbens et al., "Gastric and Duodenal Ethanol Concentrations after intake of Alcoholic Beverages in Postprandial Conditions," Molecular Pharmaceutics, (2017) 14(12):4202-4208.
Scharf, M. B., et al., "GHB—New Hope for Narcoleptics?" Biol Psychiatry (1989); 26 (4): 329-330.
Scrima, L., et al., "Narcolepsy." New England J. Med. (1991); 324 (4): 270-272.
Seno and Yamabe. "The Rheological Behavior of Suspensions of Ion-exchange Resin Particles." Bulletin of the Chemical Society of Japan (1966); 39.4: 776-778.
Shah et al., "In vitro Dissolution Profile Comparison—Statistics and Analysis of the Similarity Factor, f2," Pharm Research, (1998) 15(6):889-896.
Singh et al. "Ion exchange resins: drug delivery and therapeutic applications." Fabad J. Pharm. Sci (2007); 32: 91-100.
Srikanth et al., "Ion-exchange resins as controlled drug delivery carriers." Journal of Scientific Research (2010); 2.3: 597-611.
Takka and Gürel. "Evaluation of chitosan/alginate beads using experimental design: formulation and in vitro characterization." AAPS PharmSciTech (2010); 11.1: 460-466.
The Dow Chemical Company, Product Data Sheet for AMBERLITE™ IRN78 Resin. Form No. 177-02230-0311, Rev. 0, 3 pages.
Thorpy, M.J., "Recently Approved and Upcoming Treatments for Narcolepsy," CNS Drugs (2020) 34:9-27.
Transcript of a Markman Hearing, dated Apr. 26, 2012, in the case of *Jazz Pharmaceuticals, Inc.*, Plaintiff, v. *Roxane Laboratories, Inc.*, Defendant (United States District Court for the District of New Jersey, Civil 106108 ES), (Apr. 26, 2012).
Turnberg, L.A. "Abnormalities in intestinal electrolyte transport in congenital chloridorrhoea." Gut. (1971); 12(7): 544-551.
U.S. Department of Health and Human Services et al., "Dissolution Testing of Immediate Release Solid Oral Dosage Forms," Food and Drug Administration, CDER, Aug. 1997, 17 pages.
U.S. Department of Health and Human Services et al., "Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations", Food and Drug Administration, CDER, Sep. 1997, 27 pages.
Unknown author, title: definition of biotransformation; Medical dictionary; downloaded Jun. 21, 18 (Year: 2018), 3 pages.
Walden et al., "The Effect of Ethanol on the Release of Opioids 30 from Oral Sustained-Release Preparations," Drug Development and Industrial Pharmacy, 2007, 33:10,1101-1111.
Wermuth (Ed.), The Practice of Medicinal Chemistry, Academic Press, Third Edition, "Preparation of Water-Soluble Compounds Through Salt Formulation," Chapter 37, 2008, p. 758, 6 pages.
World Health Organization, "Annex 7: Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability," WHO Expert Committee on Specifications for Pharmaceutical Preparations Fortieth Report, pp. 347-390, 2006, retrieved from http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937_eng.pdf#page=359.
Zheng (Ed.), "Formulation and Analytical Development for Low-Dose Oral Drug Products," John Wiley & Sons, Inc., Hoboken, New Jersey, Table 4.1, p. 65, 2009, 3 pages.
Arena, C., et al., "Absorption of Sodium Gamma-Hydroxybutyrate and its Prodrug Gamma-Butyrolactone: Relationship Between In Vitro Transport and In Vivo Absorption," Journal of Pharmaceutical Sciences, 1980, 69(3): 356-358.
Bédard, M.A., et al., "Nocturnal Gamma-Hydroxybutyrate—Effect on Periodic Leg Movements and Sleep Organization of Narcoleptic Patients", Clin Neuropharmacol., 1989, 12(1): 29-36.
Berner, Jon E., "A Case of Sodium Oxybate Treatment of Tardive Dyskinsela and Bipolar Diorder," J. Clin Psychiatry, 2008, 69: 862.
Berthier, M, et al., "Possible Involvement of a Gamma-Hydroxybutyric Acid Receptor in Startle Disease", Acta Paediatr, 1994, 83(6): 678-680.
Broughton, Roger, et al., "The Treatment of Narcolepsy-Cataplexy with Nocturnal Gamma-Hydroxybutyrate", Le Journal Canadien des Sciences Neurologiques, 1979, 6(1): 285-289.
Erowid, "Gamma-hydroxybutyrate (GHB) Basic Synthesis Procedure," http://www.crowid.ondchemicals/ghb/ghb svnthcsis.shtm (as downloaded on Aug. 8, 2013).
European Patent Office, European Search Report for European Application Serial No. 03075658.9, dated Apr. 11, 2003, 5 pg.
Ferrara, S.D., et al., "Pharmacokinetics of Gamma-Hydroxybutyric Acid in Alcohol Dependent Patients After Single and Repeated Oral Doses", Br. J. Clin. Pharmaca., 1992, 34(3): 231-235.
Ferris, Trevor J., et al., "Synthesis, characterisation and detection of gamma-hydroxybutyrate salts", Forensic Science International, 2012, 216: 158-162.
Fides, "Solutions of 4-hydroxybutyric acid salts for injection," Chem Abstract ES302338, Laboratorio M. Cuatecases, S.A., 2011, 2 pp.
Frucht, S.J., et al., "A Single-Blind, Open-Label Trial of Sodium Oxybate for Myoclonus and Essential Tremor," Neurology, 2005, 65: 1967-1970.
Gallimberti, L., et al., "Gamma-Hydroxybutric Acid in the Treatment of Alcohol Dependence: A Double-Blind Study", Alcohol Clin. Exp. Res., 1992, 16(4): 673-676.
Gallimberti, L., et al., "Gamma-hydroxybutyric Acid for Treatment of Alcohol Withdrawal Syndrome", Clinical Pharmacology, 1989, 2(8666): 787-789.
Geekwench et al., "Title: Does anyone know why Jazz choose to make sodium oxybate?", Sep. 14, 2010; downloaded from http://www.talkaboutsleep.com/message/boards/topic/does-anybody-know-why-jazz-chose-to-make-sodium-oxybate/#sthash.no0PSCkL.dpuf on Jan. 21, 2015.
Geekwench et al., "Title: Does anyone know why Jazz choose to make sodium oxybate?", Sep. 14, 2010; downloaded from http://www.talkaboutsleep.com/message-boards/topic/does-anybody-know-why-jazz-chose-to-make-sodium-oxybate/ on Nov. 13, 2017 (30 pages).

(56) **References Cited**

OTHER PUBLICATIONS

Gerra, G., et al., "Flumazenil effects on growth hormone response to gammahydroxybutyric acid", Int Clin Psychopharmacol., 1994, 9(3): 211-215.
Gessa, G.L., "Gamma-Hydroxybutyric Acid in the Treatment of Alcohol Dependence", Clin. Neuropharm., 15 Suppl. 1, Pt. A, (1992), 303a-304a.
Gessa, Gian Luigi, et al., "Gamma-hydroxybutyric acid (GHB) for treatment of ethanol dependence", European Neuropsychopharmacology, 1993, 3(3): 224-225.
Grove-White, I.G., et al., "Critical Flicker Frequency after Small Doses of Methohexitone, Diazepam and Sodium 4-Hydroxybutyrate", Brit. J. Anaesth, 1971, 43(2): 110-112.
Grove-White, I.G., et al., "Effect of Methohexitone, Diazepam and Sodium 4-Hydroxybutyrate on Short-Term Memory", Brit. J. Anaesth., 1971, 43: 113-116.
Hasenbos, M A, "Anaesthesia for bullectomy. A technique with spontaneous ventilation and extradural blockade", Anaesthesia, 1985, 40(10): 977-980.
Hoes, M.J.A.J.M., et al., "Gamma-hydroxybutyric acid as hypnotic. Clinical and pharmacokinetic evaluation of gamma-hydroxybutyric acid as hypnotic in man", Encephale, 1980, 6(1): 93-99.
International Searching Authority, "International Search Report, dated Apr. 15, 2014, for Internaitonal Patent Application No. PCT/US2013/074954".
International Searching Authority, "Written Opinion, dated Apr. 15, 2014, for International Patent Application No. PCT/US2013/074954".
International Searching Authority, International Search Report and Written Opinion, dated Jun. 27, 2018, for International Patent Application No. PCT/EP2018/056745 (12 pages).
International Searching Authority, International Search Report for International Application Serial No. PCT/US99/30740, dated Jul. 21, 2000, 1 pg.
Jazz Pharmaceuticals, Inc., "XYREM® (sodium oxybate) oral solution Prescribing Information," XYREM® US Package Insert available at http://pp.jazzpharma.com/pi/xyrem.en.USPI.pdf (downloaded Sep. 12, 2017).
Jurkovich, Patti, Amendment filed in response to Written Opinion, International Application Serial No. PCT/US99/30740, filed Feb. 16, 2001, 9 pg.
Laborit, H., "Gamma-Hydroxybutyrate, Succinic Semialdehyde and Sleep," Laboratoire d'Eutonologie, 1973, 8: 257-274.
Ladinsky, Herbert, et al., "Mode of Action of Gamma-Butyrolactone on the Central Cholinergic System," Naunyn-Schmiedeberg's Arch. Pharmacal., 1983, 322: 42-48.
Lammers, G.J., et al., "Gammahydroxybutyrate and Narcolepsy: A Double-Blind Placebo-Controlled Study," Sleep, 1993, 16(3): 216-220.
Lapierre, O., et al., "The Effect of Gamma-Hydroxybutyrate on Nocturnal and Diurnal Sleep of Normal Subjects: Further Considerations on REM Sleep-Triggering Mechanisms," Sleep, 1990, 13(1): 24-30.
Lee, C.R., "Evidence for the Beta-Oxidation of Orally Administered 4-Hydroxybutyrate in Humans", Biochemical Medicine, 1977, 17(3): 284-291.
Lettieri, John, et al., "Improved Pharmacological Activity via Pro-Drug Modification: Comparative Pharmacokinetics of Sodium Gamm-Hydroxybutyrate and Gamma-Butyrolactone", Research Communications in Chemical Pathology and Pharmacology, 1978, 22(1): 107-118,.
Lynch, M., "Malic Acid", The Handbook of Pharmaceutical Excipients, 2nd Ed., 1994, 63 3: 285-286.
Mamelak, M., et al., "Sleep-Inducing Effects of Gammahydroxybutyrate", The Lancet, 1973, 2(7824): 328-329.
Mamelak, Mortimer, "Gammahydroxybutyrate: An Endogenous Regulator of Energy Metabolism", Neuroscience and Biobehavioral Reviews, 1989, 13(4): 187-198.
Mamelak, Morty, et al., "The Effects of Gamma-Hydroxybutyrate on Sleep", Biological Psychiatry, 1977, 12(2): 273-288.
Morrison, Robert T., et al., "Organic Chemistry", Chapter 20: "Functional Derivatives of Carboxylic Acids," 3rd Edition, 1973, pp. 658-700.
Nema, Sandeep, et al., "Excipients and Their Use in Injectable Products", PDA J. Pharm. Sci. Technol, 1997, 51(4): 166-171.
Neuman, Ariel, "GHB's Path to Legitimacy: An Administrative and Legislative History of Xyrem", paper submitted to Harvard Law School, 2004, 1-39.
Ondo, William G., et al., "Sodium Oxybate for Excessive Daytime Sleepiness in Parkinson Disease," Arch. Neural., 2008, 65(10): 1337-1340.
Palatini, P., et al., "Dose-Dependent Absorption and Elimination of Gamma-Hydroxybutyric Acid in Healthy Volunteers", Eur. J. Clin Pharmacal., 1993, 45(4): 353-356.
Roth, R. H., et al., "Gamma-Butyrolactone and Gamma-Hydroxybutyric Acid-II. The Pharmacologically Active Form", J. Neuropharmacol. 1966, 5: 421-428.
Roth, Robert H., et al., "Gamma-Butyrolactone and Gamma-Hydroxybutyric Acid-I, Distribution and Metabolism", Biochemical Pharmacology, 1966, 15: 1333-1348.
Russel, I. Jon, et al., "Sodium Oxybate Relieves Pain and Improves Function in Fibromyaligia Syndrome," Arthritis. Rheum , 2009, 60: 299-309.
Scharf et al., "Effect of Gamma-Hydroxybutyrate on Pain, Fatigue, and the Alpha Sleep Anomaly in Patients with Fibromyalgia. Preliminary Report", The Journal of Rheumatology, 25(10): 1986-1990 (1998).
Scharf, M.B., et al., "The Effects and Effectiveness of Gamma-Hydroxybutyrate in Patients with Narcolepsy", J. Clin. Psychiatry, 1985, 46(6): 222-225.
Scharf, Martin B., et al., The Effects of Sodium Oxybate on Clinical Symptoms and Sleep Patterns in Patients with Fibromyalgia, J. Rheumatol, 2003, 30(5): 1070-1074.
Scrima, et al., "Effect of High Altitude on a Patient with Obstructive Sleep Apnea", Sleep Research, Abstract, 1987, 16: 427.
Scrima, et al., "Effects of Gamma-Hydroxybutyrate (GHB) on Narcolepsy-Cataplexy Symptoms and MSLT Results in Male and Female Patients", Association of Professional Sleep Societies, Abstract, 1988, 251.
Scrima, et al., "Gamma-Hydroxybutyrate Effects on Cataplexy and Sleep Attacks in Narcoleptics", Sleep Research, Abstract, 1987, 16: 134.
Scrima, L, et al., "Efficacy of Gamma-Hydroxybutyrate Versus Placebo in Treating Narcolepsy-Cataplexy: Double-Blind Subjective Measures", Biol. Psychiatry, 1989, 26(4): 331-343.
Scrima, L. et al., "Effect of Gamma-Hydroxybutyrate on a Patient with Obstructive Sleep Apnea," Sleep Research, Abstract, 1987, 16: 137.
Scrima, Lawrence, et al., "The Effects of Gamma-Hydroxybutyrate on the Sleep of Narcolepsy Patients: A Double-Blind Study", Sleep, 1990, 13(6): 479-490.
Sériès, F., et al., "Effects of Enhancing Slow-Wave Sleep by Gamma-Hydroxybutyrate on Obstructive Sleep Apnea", Am. Rev. Respir. Dis., 1992, 145(6): 1378-1383.
Snead, O. Carter et al., "Ontogeny of Gamma-Hydroxybutyric Acid. I. Regional Concentration in Developing Rat, Money and Human Brain," Brain Res., 1981, 227(4): 579-589.
Snead, O. Carter, "Gamma-Hydroxybutyrate Model of Generalized Absence Seizures: Further Characterization and Comparison with Other Absence Models," Epilepsia, 1988, 29(4): 361-368.
Stock, Günter, et al., "Increase in Brain Dopamine after Axotomy or Treatment with Gammahydroxybutyric Acid Due to Elimination of the Nerve Impulse Flow", Naunyn-Schmiedeberg's Arch, Pharmacal., 1973, 278(4): 347-361,.
Strong, A. J., "Gamma-Hydroxybutyric Acid and Intracranial Pressure", The Lancet, 1984, 1(8389): 1304.
Suner, S., et al., "Pediatric Gamma Hydroxybutyrate Intoxication", Acad. Emerg. Med., 1997, 4(11): 1041-1045.
Tunnicliff, Godfrey, "Sites of Action of Gamma-Hydroxybutyrate (GHB)—A Neuroactive Drug with Abuse Potential", Clinical Toxicology, 1997, 35(6): 581-590.

(56) **References Cited**

OTHER PUBLICATIONS

United States District Court, "Opinion," *Jazz Pharmaceuticals, Inc.* v. *Roxane Laboratories, Inc.*, Markman Hearing, No. 10-6108 (ES), (Sep. 14, 2012), 43 pg.

United States District Court, "Order," *Jazz Pharmaceuticals, Inc.* v. *Roxane Laboratories, Inc.*, Markman Hearing, No. 10-6108 (ES), (Sep. 14, 2012), 1 pg.

United States Pharmacopeia (USP), Pharmaceutic Ingredients, 23/NF18, 1995, p. 2205.

Van Den Bogert, et al., "Placentatransfer of 4-Hydroxybutyric Acid in Man", Anaesthesiology and Intensive Care Medicine, 1978, 110: 55-64.

Vickers, M.D., "Gammahydroxybutyric Acid", Int. Anesth. Clinic, 1969, 7(1): 75-89.

Vogel et al., 2018, "Toxicologic/transport properties of NCS-382, a γ-hydroxybutyrate (GHB) receptor ligand, in neuronal and epithelial cells: Therapeutic implications for SSADH deficiency, a GABA metabolic disorder," Toxicol In Vitro, 46:203-212 (Epub 2017),.

Yamada, Y., et al., "Effect of Butyrolactone and Gamma-Hydroxybutyrate on the EEG and Sleep Cycle in Man", Electroenceph. clin. Neurophysiol., 1967, 22: 558-562.

Chen et al., "Pharmacokinetics, relative bioavailability and food effect of JZP-258 and sodium oxybate: results of two phase 1, open-label, randomised crossover studies in healthy volunteers," Sleep Medicine, Abstracts, 2019, vol. 64, pp. S65-S66.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2021/019024, dated Jun. 2, 2021, 10 pages.

Jha, M.K, "Modified release formulations to achieve the quality target product profile (QTPP)," IJPSR, 2012; vol. 3(8): 2376-2386.

Rujivipat et al., "Improved drug delivery to the lower intestinal tract with tablets compression-coated with enteric/nonenteric polymer powder blends," European Journal of Pharmaceutics and Biopharmaceutics (2010) 76: 486-492.

Non-Final Office Action dated Aug. 25, 2021, for U.S. Appl. No. 17/222,579, 14 pages.

* cited by examiner

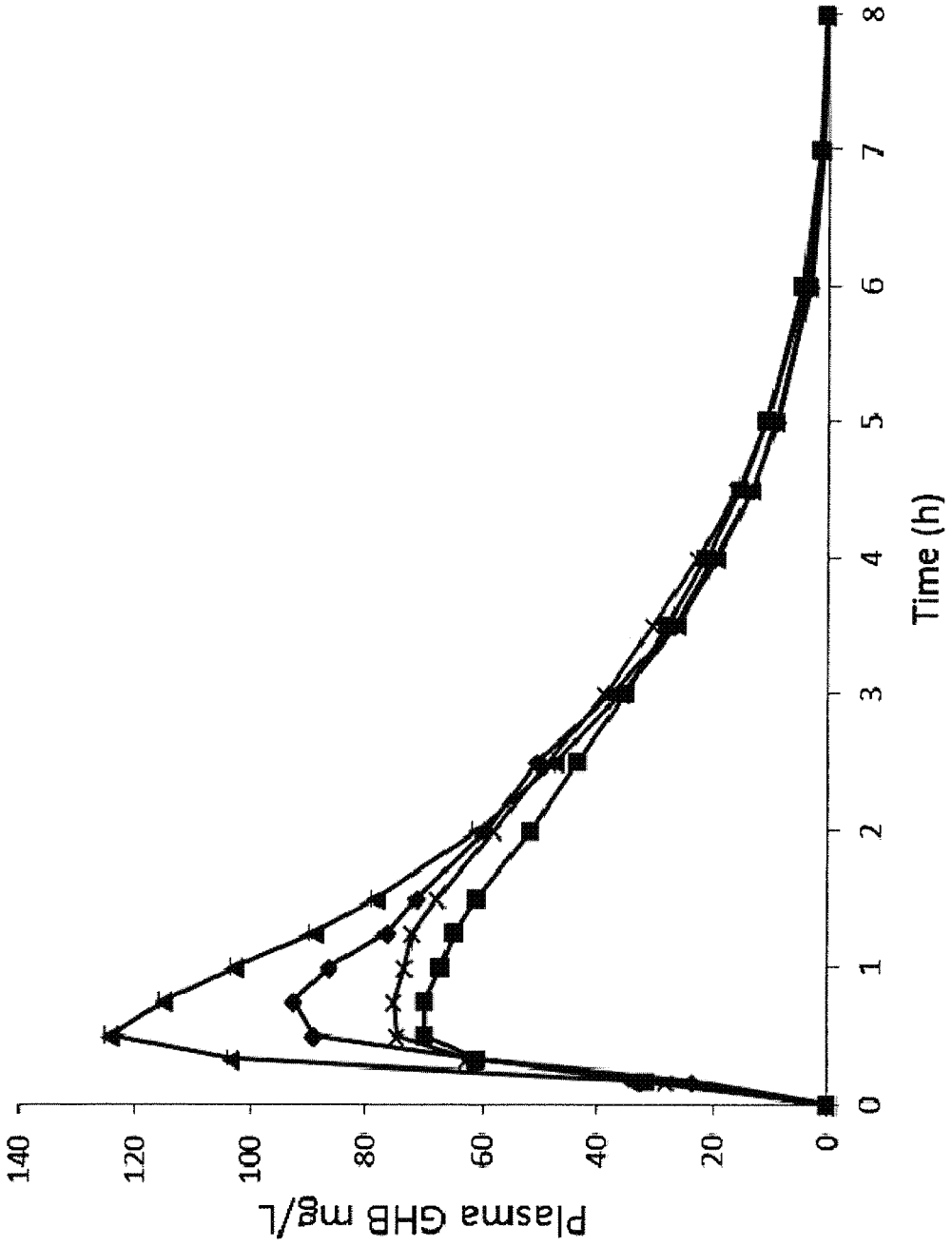


FIG. 1

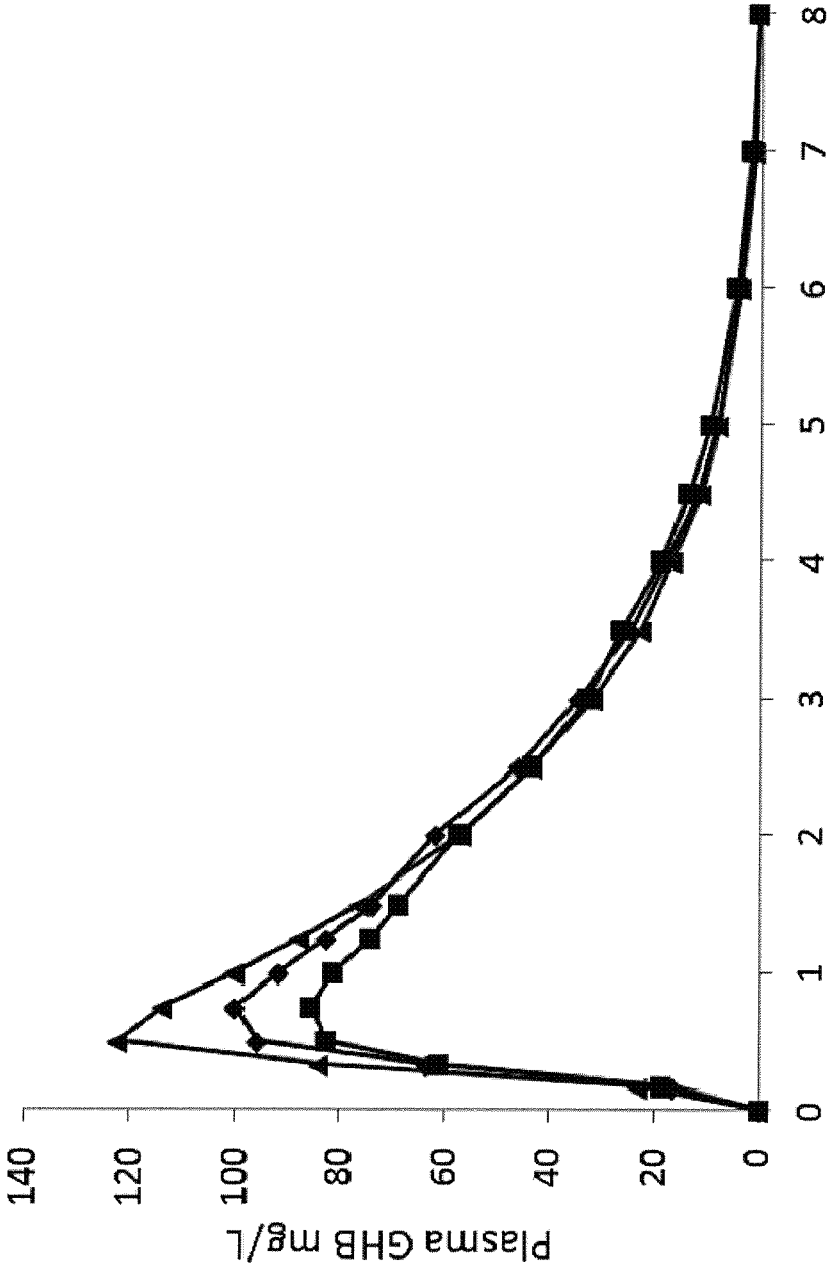


FIG. 2

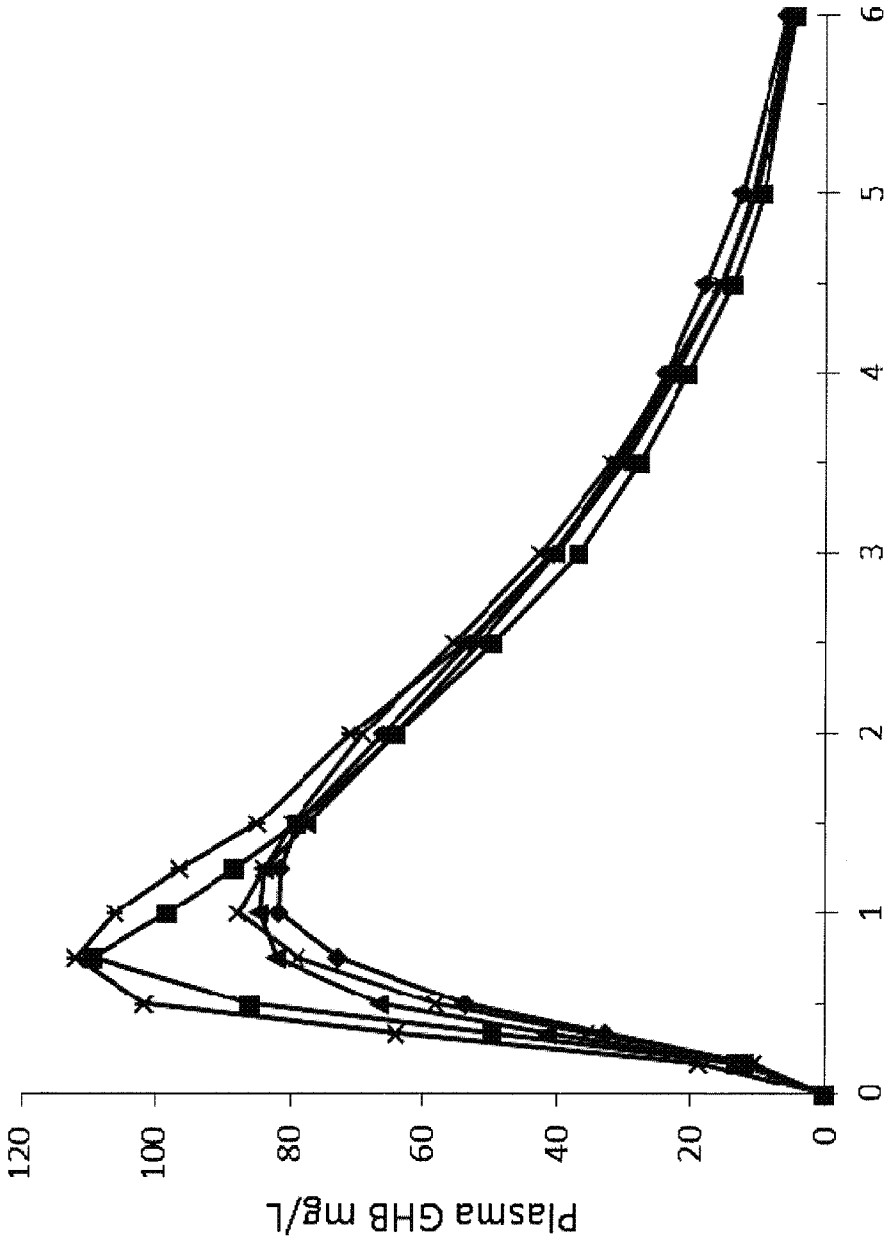


FIG. 3

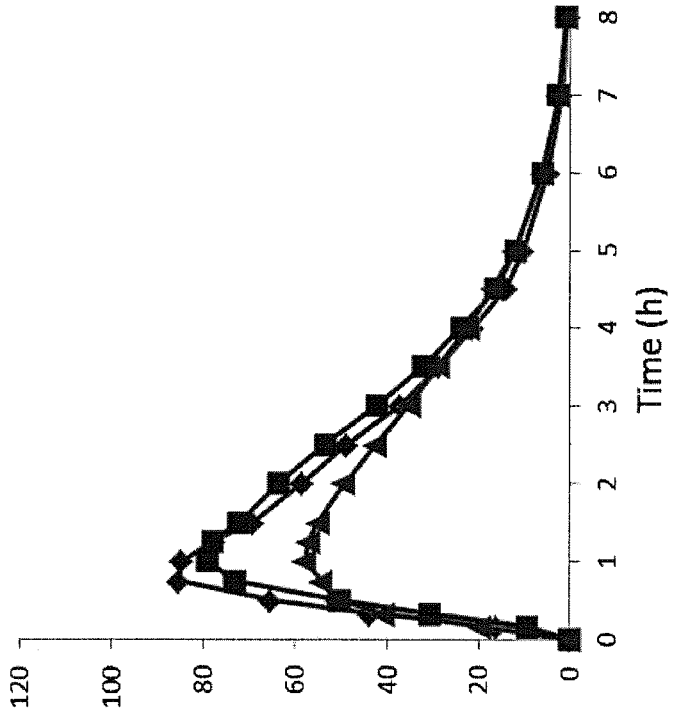

120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8
Time (h)

FIG. 4B

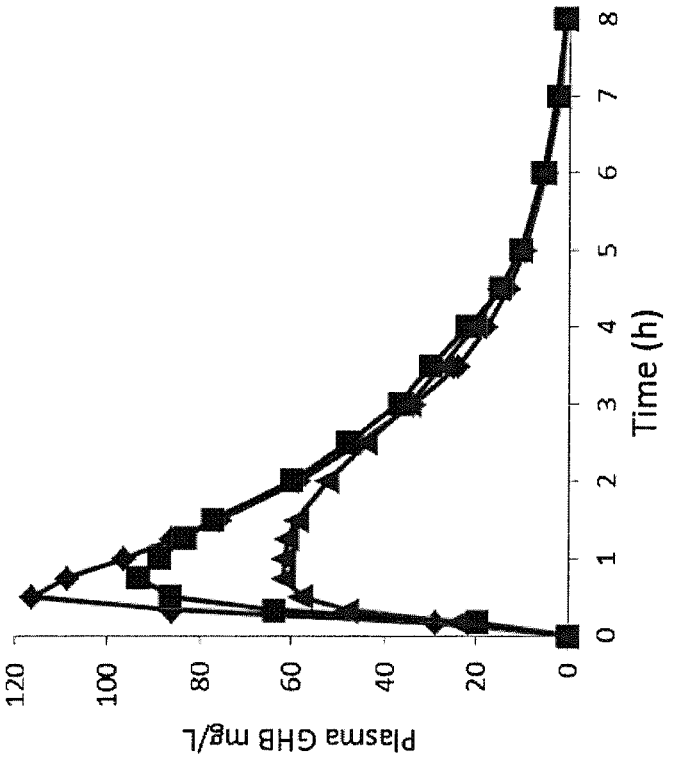

Plasma GHB mg/L
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8
Time (h)

FIG. 4A

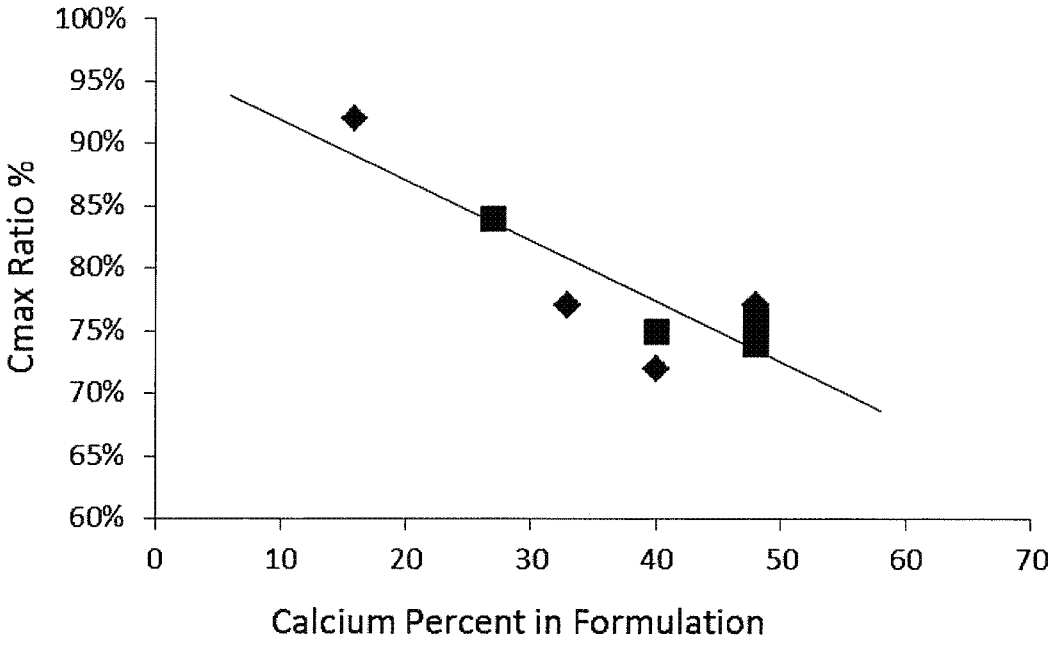

100%
95%
90%
85%
80%
75%
70%
65%
60%
Cmax Ratio %
0
10
20
30
40
50
60
70
Calcium Percent in Formulation

FIG. 5A

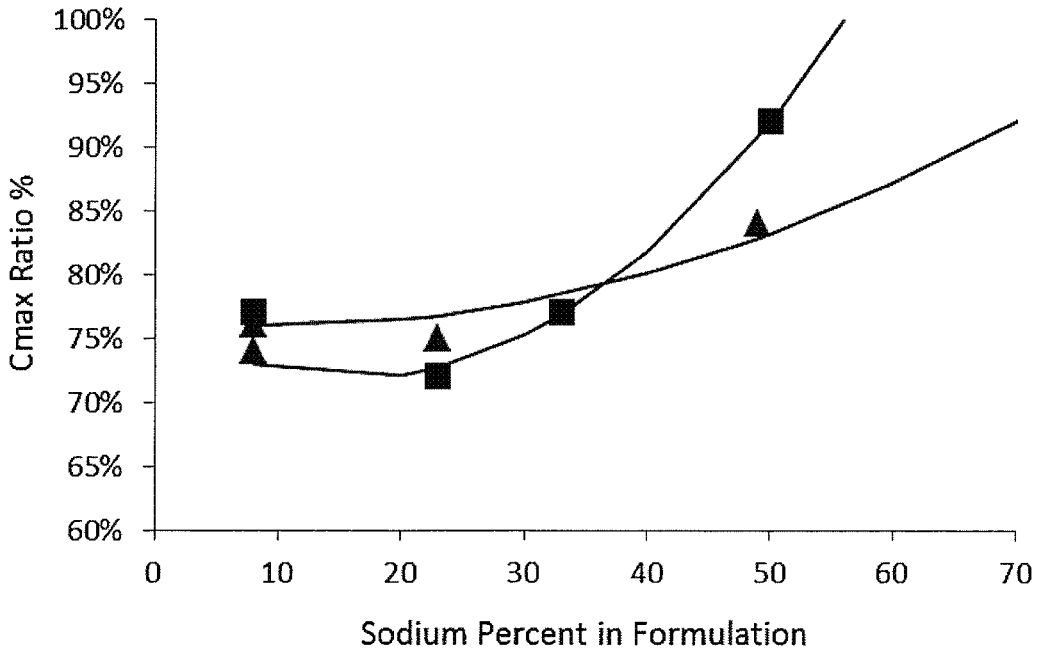

100%
95%
90%
85%
80%
75%
70%
65%
60%
Cmax Ratio %
0
10
20
30
40
50
60
70
Sodium Percent in Formulation

FIG. 5B

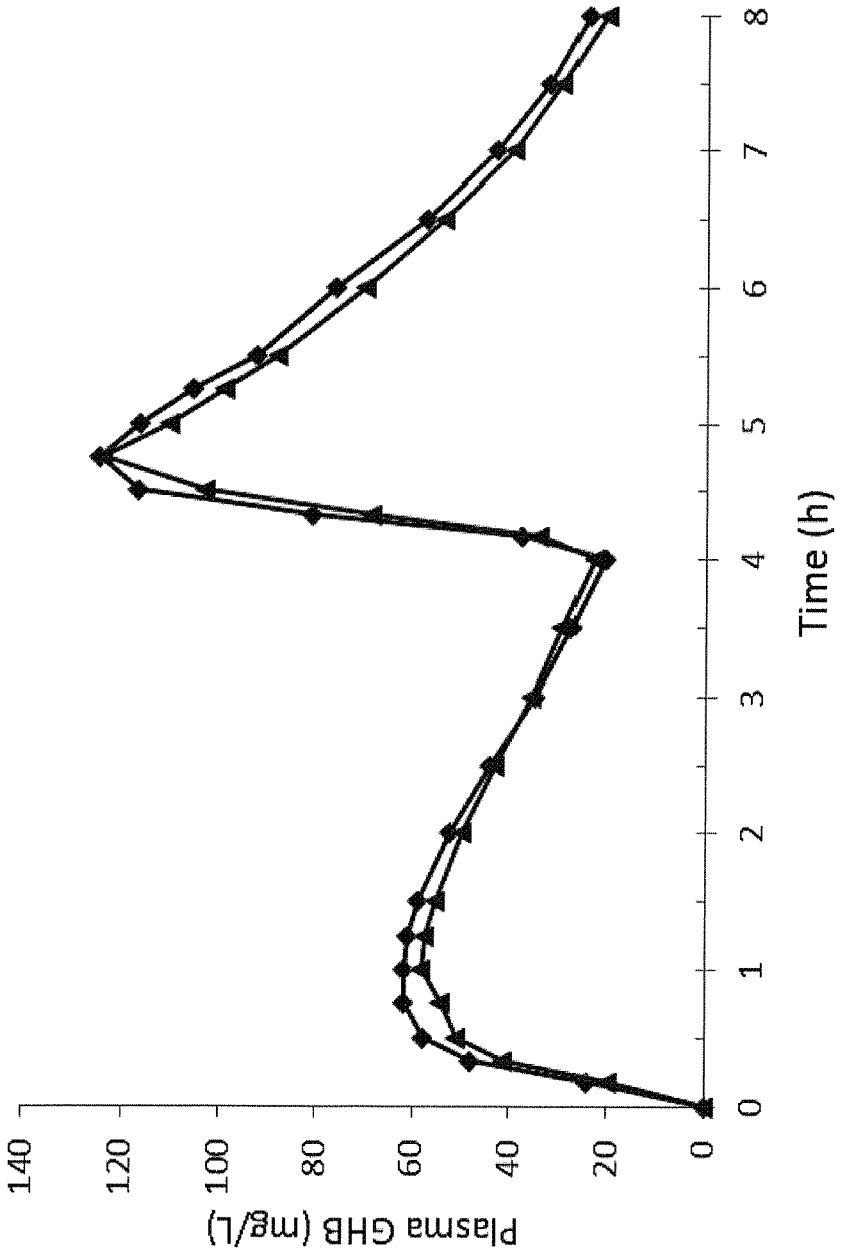


FIG. 6

GAMMA-HYDROXYBUTYRATE COMPOSITIONS AND THEIR USE FOR THE TREATMENT OF DISORDERS

1. CROSS REFERENCE

This application is a continuation of U.S. patent application Ser. No. 16/575,213, filed Sep. 18, 2019, which is a continuation of U.S. patent application Ser. No. 15/709,262, filed Sep. 19, 2017, now abandoned, which claims the benefit of U.S. Provisional Patent Application No. 62/473,232, filed Mar. 17, 2017, the content of each of which is incorporated herein by reference in its entirety.

2. FIELD OF THE INVENTION

Provided herein are pharmaceutical compositions and formulations comprising salts of gamma-hydroxybutyrate (GHB). In one embodiment, the salts encompass more than one type of cation. Also provided herein are methods of making the pharmaceutical compositions and formulations, and methods of the treatment of disorders including fibromyalgia and sleep disorders. Also described herein is that such pharmaceutical compositions and formulations are for treating diseases or disorders including fibromyalgia and sleep disorders. Such sleep disorders include apnea, sleep time disturbances, narcolepsy, cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

3. BACKGROUND OF THE INVENTION

Sodium oxybate (Na.GHB), commercially sold as Xyrem® (Jazz Pharmaceuticals), is approved for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Na.GHB has also been reported to be effective for relieving pain and improving function in patients with fibromyalgia syndrome (See Scharf et al., 2003, *J. Rheumatol.* 30: 1070; Russell et al., 2009, *Arthritis. Rheum.* 60: 299), and in alleviating excessive daytime sleepiness and fatigue in patients with Parkinson's disease, improving myoclonus and essential tremor, and reducing tardive dyskinesia and bipolar disorder (See Ondo et al., 2008, *Arch. Neural.* 65: 1337; Frucht et al., 2005, *Neurology* 65: 1967; Berner, 2008, *J. Clin. Psychiatry* 69: 862).

Xyrem®, for use with patients with narcolepsy, is a chronically used product which requires high levels of the drug. The amount of sodium intake from the drug significantly increases the daily sodium intake for patients, which is undesirable for patients with hypertension, heart disease, renal disease or at risk of stroke.

Since Xyrem® is administered to a broad population, there is a need for GHB formulations that minimize the undesirable side effects of the sodium, particularly in patients with hypertension, heart disease, renal disease or at risk of stroke, yet provide additional health benefits from the presence of the other salts. It is desirable that such modified formulations provide good solubility, stability and purity in order to provide safe, effective and consistent doses to patients, and also display acceptable pharmacodynamic and pharmacokinetic properties. See U.S. Pat. Nos. 8,591,922; 8,901,173; and 9,132,107; which are incorporated by reference in their entireties.

4. SUMMARY OF THE INVENTION

Provided herein are pharmaceutical compositions and formulations comprising salts of gamma-hydroxybutyrate

("GHB") which are useful in the treatment of conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, excessive daytime sleepiness (EDS) cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

One embodiment, as provided herein, is a GHB formulation with a reduction in sodium content. Another embodiment, as provided herein, is a GHB formulation with a reduced sodium content and which is bioequivalent to Xyrem®. In certain embodiments, the reduction in sodium content involves use of other cations such as potassium, calcium, magnesium, and others.

For convenience in comparing various salt compositions at the same oxybate or GHB molar dose, compositions expressed as percentages in this application refer to molar equivalent percentage (% molar equivalents) of each salt of oxybate or GHB. This is usually close to, but not the same as, a composition that would be expressed as wt/wt %. As used herein, the terms "oxybate" and "GHB" are used interchangeably.

Accordingly, in one aspect, provided herein are pharmaceutical compositions and formulations comprising salts of GHB. In one embodiment, the formulation is a pharmaceutical composition of GHB comprising a mixture of two or more salts of GHB, wherein the mixture comprises at least 50% of a sodium salt of gamma-hydroxybutyrate (Na.GHB), and wherein the mixture further comprises one or more of a potassium salt of gamma-hydroxybutyrate (K.GHB) and a calcium salt of gamma-hydroxybutyrate ($Ca.(GHB)_2$). In certain embodiments, the Na.GHB salt is present in the mixture in about 50%, and up to 55%, 60%, 70% or 80%. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of a magnesium salt of gamma-hydroxybutyrate ($Mg.(GHB)_2$).

In another embodiment the pharmaceutical composition is given to the patient in an aqueous solution with a volume of between 25 and 100 mL, 25 and 75 mL, or 55 and 65 mL.

In another embodiment, the pharmaceutical composition, when administered to a patient, is bioequivalent to the average maximum GHB plasma concentration (Cmax) and the average maximum GHB plasma area under the curve (AUC) of the Cmax of Na.GHB within 80% to 125%.

In another embodiment, the pharmaceutical composition comprises a mixture of three salts of GHB, wherein the mixture comprises at least 50% of Na.GHB, and further comprises K.GHB and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises between 50 and 60% of Na.GHB, and further comprises between 20 and 40% K.GHB, and between 10 and 20% $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises about 50% of Na.GHB, 34% K.GHB, and 16% $Ca.(GHB)_2$ for each GHB salt.

In another embodiment, the pharmaceutical compositions and/or formulations disclosed herein can be used to treat a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure.

In another embodiment, the pharmaceutical compositions disclosed herein comprise less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture

of two or more GHB salts, the mixture comprising between 40% to 50% Na.GHB and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical compositions disclosed herein do not comprise a substantial amount Ca.$(GHB)_2$) or Mg.$(GHB)_2$.

In another embodiment, the pharmaceutical composition comprises about 8% Na.GHB, 23% K.GHB, 48% Ca.$(GHB)_2$ and 21% Mg.$(GHB)_2$. In certain embodiments, this pharmaceutical composition can be used to treat the diseases or conditions listed above.

In another embodiment, the pharmaceutical compositions and/or formulations disclosed herein, when administered to a patient, have a lower average maximum GHB plasma concentration (Cmax) than the Cmax of Na.GHB.

Xyrem®, as disclosed herein, is a commercially sold product comprised of 100% sodium oxybate (Na.GHB), and is prescribed for twice nightly use for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Accordingly, in another aspect, provided herein is a first dose of a first pharmaceutical composition and/or formulation having a Na.GHB of less than 50% and a second dose of a second pharmaceutical composition and/or formulation having a Na.GHB above 50%. Another embodiment has the doses in reverse order and a further embodiment uses similar doses of either formulation. In certain embodiments, the first dose can be administered within 4 hours of eating and produces a GHB Cmax lower than the Cmax of Na.GHB, but may have less of a food effect.

In another aspect, the pharmaceutical compositions and formulations provided herein can be used to treat a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In one embodiment, the formulations and pharmaceutical compositions provided herein can be used to treat conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

The pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g. Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In certain embodiment, the formulations and pharmaceutical compositions disclosed herein are used in a method of treating conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In another aspect, provided herein are methods of treating a disease or condition in a patient that is suitable for treatment with GHB, comprising administering to the patient the pharmaceutical compositions and formulations disclosed herein. In certain embodiments, the disease or condition is selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In certain embodiments, the disease or condition is elected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In another embodiment, methods of treatment disclosed herein comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering to a patient having a disease or condition that is suitable for treatment with GHB the first dose; and (iv) orally administering to the patient the second dose within 2.5 to 4 hours following the first dose.

The pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition in a patient that is suitable for treatment with GHB, comprising administering to the patient the pharmaceutical compositions and formulations disclosed herein.

In certain embodiments, the pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition in a patient further comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering to a patient having a disease or condition that is suitable for treatment with GHB the first dose; and (iv) orally administering to the patient the second dose within 2.5 to 4 hours following the first dose.

In other aspects, provided herein are methods of making the pharmaceutical compositions disclosed herein.

5. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows the plasma GHB concentration vs time for Formulation "O" (8% Na.GHB, 23% K.GHB, 48% Ca.(GHB), and 21% Mg.$(GHB)_2$) compared to Xyrem® ("X") given in either the fed or fasted state (, Xyrem® fasted; , Formulation "O" fasted; Xyrem® fed; , Formulation "O" fed). The objective was to characterize bioequivalence of Formulation "O" to Xyrem®.

FIG. **2** shows the plasma GHB concentration vs time for blends of Formulation "O" and Xyrem® ("X") in proportions of 100% Xyrem®, 44% Xyrem®, and 17% Xyrem®, respectively (, fasted 4.5 g "X"; , fasted 2.5 g "O"+2.0 g "X"; , fasted 3.75 g "O"+0.75 g "X"). The

objective was to determine how much sodium (or Xyrem®) would be required to achieve bioequivalence in the fasted state.

FIG. **3** shows the plasma GHB concentration vs time for various mixed oxybate salt formulations compared to Xyrem® in the fasted state where both are given at a lower volume of administration of 60 mL (, Xyrem® (100% Na); , Formulation 507D (50% Na, 34% K, 16% Ca, 0% Mg); , 507C (33% Na, 0% K, 48% Ca, 19% Mg); , 507A (33% Na, 34% K, 33% Ca, 0% Mg); , 507G (23% Na, 19% K, 40% Ca, 18% Mg)).

FIG. **4**A-**4**B compare Xyrem® and Formulation "O" when given fasted with 60 mL or 240 mL water or when given fed with 60 mL water. FIG. **4**A. (Left) Plasma GHB concentration when Xyrem® was given (fasted) with 60 mL or 240 mL water or when Xyrem® was given (fed) with 60 mL water (, fasted 240 mL; , fasted 60 mL; , fed 60 mL). FIG. **4**B (Right) Plasma GHB concentration when Formulation "O" was given (fasted) with 60 mL or 240 mL water or when Formulation "O" was given (fed) with 60 mL water (, fasted 240 mL; , fasted 60 mL; , fed 60 mL).

FIG. **5**A-**5**B show the relationship between Cmax ratio (to Xyrem®) and calcium content or sodium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume or 60 mL aqueous volume. FIG. **5**A. (Top) Relationship between Cmax ratio (to Xyrem®) and calcium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume (, Cmax, 60 mL; , Cmax, 240 mL). FIG. **5**B (Bottom) Relationship between Cmax ratio (to Xyrem®) and sodium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume (, Cmax, 60 mL; , Cmax, 240 mL).

FIG. **6** is a graph showing the expected behavior of taking separate formulations as part of an equally divided dose given 4 h apart (, 1st dose Xyrem® fed, 2nd dose Xyrem® fasted; , 1st dose Formulation "O" fed, 2nd dose Formulation 507D fasted). Formulation "O" is given initially and then formulation "507D" is given 4 h later. This is compared to Xyrem® given both times.

6. DETAILED DESCRIPTION OF THE INVENTION

Gamma-hydroxybutyrate (GHB), also known as "oxybate," is an endogenous compound with hypnotic properties that is found in human body tissues, such as the mammalian brain. In the brain, the highest GHB concentration is found in the hypothalamus and basal ganglia and GHB is postulated to function as a neurotransmitter (See Snead and Morley, 1981, *Brain Res.* 227(4): 579-89). The neuropharmacologic effects of GHB include increases in brain acetylcholine, increases in brain dopamine, inhibition of GABA-ketoglutarate transaminase and depression of glucose utilization but not oxygen consumption in the brain. GHB treatment substantially reduces the signs and symptoms of narcolepsy, i.e., daytime sleepiness, cataplexy, sleep paralysis, and hypnagogic hallucinations. In addition, GHB increases total sleep time and REM sleep, and it decreases REM latency, reduces sleep apnea, and improves general anesthesia (see, e.g., U.S. Pat. Nos. 6,472,431; 6,780,889; 7,262,219; 7,851,506; 8,263,650; 8,324,275; and 8,772,302 each of which is incorporated herein by reference in its entirety).

Xyrem® is a commercially sold product comprised of 100% sodium oxybate (Na.GHB) and is approved for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Na.GHB has also been reported to be effective for relieving pain and improving function in patients with fibromyalgia syndrome, and in alleviating excessive daytime sleepiness and fatigue in patients with Parkinson's disease, improving myoclonus and essential tremor, and reducing tardive dyskinesia and bipolar disorder. See the references that are incorporated at the end of U.S. Pat. No. 6,472,431. Further, despite a general record of safety when used as prescribed, there are risks of abuse and misuse of Xyrem® which can cause serious medical problems, including seizures, loss of consciousness, coma, and death (see, e.g., FDA product label dated Nov. 13, 2006 for NDA no. 021196, which is incorporated by reference in its entirety).

Xyrem® for use with patients with narcolepsy, is a chronically used product which requires high levels of the drug. The amount of sodium intake from the drug significantly increases the daily sodium intake for patients, which is undesirable for patients with hypertension, heart disease, renal disease or at risk of stroke. Thus, there is a need for GHB formulations with lower sodium, such as those provided herein, particularly for patients with hypertension, heart disease, renal disease or at risk of stroke, yet provide additional health benefits from the presence of the other salts.

However, the therapeutic dose of 71.4 mEq/day (9 g sodium oxybate) is sufficiently high that shifting from sodium to another cation can push limits on acceptable daily intake of other cations and potentially cause other problems for certain patients. For example, potassium has poor tolerability in solution at high doses given on an empty stomach and can also be problematic for patients with kidney impairment. Therefore, formulations which reduce or eliminate sodium without exceeding levels of concern for other cations are particularly desirable.

Xyrem® is provided as an oral solution consisting of 500 mg/mL sodium oxybate (Na.GHB) that is pH adjusted with malic acid. Xyrem® is rapidly and well absorbed when given on an empty stomach. The absolute bioavailability for 2.25 g and 4.45 g sodium oxybate doses, relative to IV administration, is 88%. See the Xyrem® Product Insert. As a result, sodium oxybate is generally considered to be a high solubility, high permeability drug. (See Yu et al., *Pharm. Res.* 19 (7) 921-925). As such, for alternative formulations of GHB, such as those comprising cations other than sodium, but having comparable solubility, bioequivalence might be expected and a pharmacokinetic evaluation waived. See 21 CFR Part 320.22 Subpart B paragraph b(3).

However, as disclosed herein, despite the apparently rapid absorption of sodium oxybate, its presentation as an aqueous solution, and the absence of any other ingredients that would be expected to modify absorption behavior, formulations having the same GHB concentration do not display pharmacokinetics equivalent to Xyrem®. Furthermore, as also disclosed herein, the pharmacokinetic behavior of such formulations appears to depend on the amount of sodium and/or other cations present, as well as the amount of water in the formulation. Accordingly, one object of the present disclosure is to provide alternative formulations of GHB which are bioequivalent to Xyrem®. Provided herein are such alternative formulations which surprisingly display the desired bioequivalence.

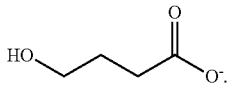
The following patents and applications referred to throughout the application are hereby incorporated by ref-

erence in their entireties for all purposes, including the following: U.S. Pat. Nos. 6,472,431; 7,895,059; 8,461,197; 8,591,922; 8,759,394; 8,771,735; 8,772,306; 8,778,301 8,778,398; 8,952,029; and 9,050,302; and U.S. Publication No. 2012/0076865.

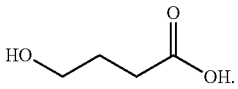
Objects, features and advantages of the methods and compositions described herein will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

6.1 Definitions

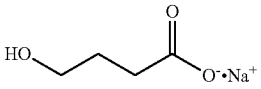
As used herein, the term "gamma-hydroxybutyrate" (GHB) or "oxybate" refers to the negatively charged or anionic form (conjugate base) of gamma-hydroxybutyric acid. Without being limited by theory, GHB is believed to have the following structure:



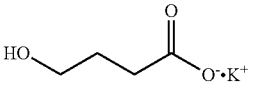
As used herein, the term "gamma-hydroxybutyric acid" refers to the protonated form (conjugate acid) of gamma-hydroxybutyrate. Without being limited by theory, gamma-hydroxybutyric acid is believed to have the following structure:



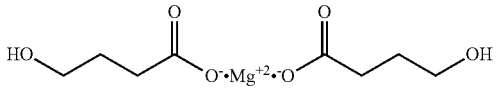
As used herein, the terms "sodium gamma-hydroxybutyrate" (Na.GHB) or "sodium oxybate" (Na.oxybate) refers to the sodium salt form of gamma-hydroxybutyric acid having the molecular weight of 126.09. Without being limited by any theory, Na.GHB is believed to have the following structure:



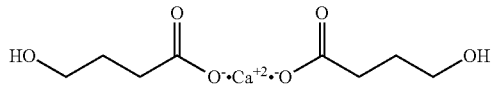
As used herein, the term "potassium gamma-hydroxybutyrate" (K.GHB) or "potassium oxybate" (K.oxybate) refers to the potassium salt form of gamma-hydroxybutyric acid having the molecular weight of 142.19. Without being limited by any theory, K.GHB is believed to have the following structure:



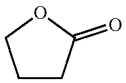
As used herein, the term "magnesium gamma-hydroxybutyrate" ($Mg.(GHB)_2$) or "magnesium oxybate" (Mg.oxybate) refers to the magnesium salt form of gamma-hydroxybutyric acid having the molecular weight of 230.50. Without being limited by theory, $Mg.(GHB)_2$ is believed to have the following structure:



As used herein, the term "calcium gamma-hydroxybutyrate" ($Ca.(GHB)_2$) or "calcium oxybate" (Ca.oxybate) refers to the calcium salt form of gamma-hydroxybutyric acid having the molecular weight of 246.27. Without being limited by theory, $Ca.(GHB)_2$ is believed to have the following structure:



As used herein, the term "gamma-butyrolactone" (GBL) refers to a colorless oily liquid. Without being limited by theory, GBL is believed to have the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

The terms "treat," "treating" or "treatment," as used herein, refer to a method of alleviating or abrogating a disease and/or its attendant symptoms.

As used herein, the term "about" or "approximately" means an acceptable error for a particular value as determined by those skilled in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value.

The term "substantial amount" shall mean over 1%.

By "pharmaceutically acceptable" it is meant the active ingredient, cation, salt, diluent, excipient or carrier must be compatible with the other ingredients of the formulation and not unduly deleterious, for example, that the active ingredient, cation, salt, diluent, excipient or carrier does not produce an adverse, allergic or other untoward reaction, when administered to an animal, or a human, as appropriate.

The term "salt" or "salts," as used herein, refers to a compound formed by the interaction of an acid and a base, the hydrogen atoms of the acid being replaced by the positive ion or cation of the base. Pharmaceutically acceptable salts, include inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as malic, acetic, oxalic, tartaric, mandelic, and the like. Salts formed can also be derived from inorganic bases such as, for example, sodium, potassium, silicates, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. In certain preferred embodiments, the salt is formed from an

inorganic base that is a metal, for example, an alkali metal, such as lithium, potassium, sodium, or the like, an alkaline earth metal, such as magnesium, calcium, barium, or the like, or aluminum or zinc. Other salts may comprise ammonium. Alkali metals, such as lithium, potassium, sodium, and the like, may be used, preferably with an acid to form a pH adjusting agent. Examples of pharmaceutically acceptable base addition salts include those derived from inorganic bases like sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, or ammonium hydroxide, and the like (See, e.g., Berge et al., 1977, *J. Pharm. Sci.* 66: 1).

As used herein, the terms "salt of GHB" or "salts of GHB," as used herein, refer to a compound formed by the interaction of gamma-hydroxybutyric acid (the conjugate acid of GHB) with a base, for example, NaOH, KOH, $Mg(OH)_2$, and $Ca(OH)_2$, and the like, the hydrogen atoms of the acid being replaced by the positive ion or cation of the base. Such salts may include, for example, Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$, and the like. It will be understood by those skilled in the art that such salts may be in solid form, or such salts may be in partially or fully solvated form, for example, as when dissolved in an aqueous medium. It will be further understood by those skilled in the art, that, depending on the solubility of the salt in the aqueous medium, that the salt may be present in the aqueous medium as solvated cation(s) and anion(s), or as a precipitated solid, as illustrated below for the solubility equilibrium of $Ca.(GHB)_2$:

$$Ca\bullet(GHB)_{2(s)} \overset{H_2O}{\rightleftharpoons} Ca^{+2}{}_{(aq)} + 2\,(GHB)^{-}{}_{(aq)}$$

The terms "mixture of salts" or "salt mixture," as used herein, refers to salts of GHB where two or more different cations are present in combination with each other in a composition. Such mixtures of salts may include, for example, two or more salts selected from the group consisting of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$.

Xyrem® contains 500 mg/mL Na.GHB. When referring to a mixture of GHB salts with different cations, the concentration in mg/mL will vary between formulations and/or pharmaceutical compositions of the same GHB strength. As used herein, a GHB concentration of 409 mg/mL is equivalent to the GHB content in 500 mg/mL of Na.GHB.

The term "wt/wt %," are used herein, refers to the normalized weight percent of a particular salt in a salt mixture. A sample calculation of wt/wt % is provided in Example 1 of the present disclosure.

The term "wt/wt % ratio," as used herein, refers to the ratio of wt/wt % values in a mixture of salt. For example, where the salts Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$ are present in a wt/wt %'s of 8%, 32%, 20% and 40%, respectively, the wt/wt % ratio of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$ in the mixture is 8%:32%:20%:40%.

The terms "% molar equivalents" and "% mol. equiv.," as used herein, refer to molar composition of salts expressed as a percent of GHB (or "oxybate") equivalents. For example, formulations and/or pharmaceutical compositions as described herein comprise mixtures with varying percentages of oxybate, expressed as % molar equivalents (% mol. equiv.) of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. Those skilled in the art will understand that as each GHB unit is considered to be one molar equivalent, the monovalent cations, Na^+ and K^+, have one molar equivalent per salt, and the divalent cations, Mg^{+2} and Ca^{+2}, have two molar equivalents per salt. A sample calculation of % mol. equiv. is provided in the Examples of the present disclosure. For convenience in comparing various salt compositions at the same oxybate molar dose, compositions expressed as percentages in this application refer to molar equivalent percentage (% molar equivalents) of each oxybate salt. This is usually close to, but not the same as, the composition that would be expressed as wt/wt %.

The term, "buffering agent," as used herein, refers to a weak acid or base used to maintain the pH of a solution near a chosen pH value after the addition of another acidic or basic compound. The function of such an agent is to prevent the change in pH when acids or bases are added to a solution. Such agents may be acids, bases, or combinations thereof.

The term, "adjusting agent," as used herein, refers to an acid or base used to alter the pH of a solution to a chosen pH value. The function of such an agent is to alter the pH of a solution to the desired value subsequent to the addition of acidic or basic compounds.

The term, "acid," as used herein, refers to a substance which accepts a share in a pair of electrons. Such substances include malic acid, citric acid, acetic acid, boric acid, lactic acid, hydrochloric acid, phosphoric acid, sulfuric acid, sulfonic acid, nitric acid, and the like.

The term, "base," as used herein, refers to a substance which shares a pair of electrons. Such substances include sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, and the like.

The term, "chemically stable," as used herein, refers to a chemical compound which is not particularly reactive in a specific environment and retains its useful properties on a timescale of its expected usefulness. Specifically, the usefulness of the compound is maintained in the presence of air, moisture, or heat. Conversely, the compound lacks chemical stability if it decomposes under the conditions of a specific environment. As used herein in certain embodiments, "chemically stable" may mean resistant to degradation of GHB into its known or unknown decomposition elements. The level of GBL that is acceptable can be up to 0.15% of the formulation as per the ICH guidelines for shelf-life determination.

The term, "microbial," as used herein, refers to a microscopic organism that comprises either a single cell, cell cluster or multicellular organism.

The term "resistant to microbial growth" or "resistant to microbial challenge," as used herein, means that the compositions or formulations meet the criteria set by the Food and Drug Administration and the U.S. Pharmacopoeia for products made with aqueous bases or vehicles, which for bacteria means not less than a 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days, and for yeast and molds, no increase from the initial calculated count at 14 and 28 days.

The term, "preservative," as used herein, refers to a naturally occurring or synthetically produced substance which can be added to food, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by chemical decomposition.

The term, "formulation," as used herein, refers to a stable and pharmaceutically acceptable preparation of a pharmaceutical composition disclosed herein.

The term, "liquid formulation," as used herein, refers to a water-based formulation, in particular, a formulation that is an aqueous solution.

The term, "low volume" or "low aqueous volume" or "reduced volume," as used herein, refers to an aqueous solution of about 100 mL or less.

The term, "volume of administration" as used here, refers to the volume of aqueous material used to ingest or swallow the formulations and/or pharmaceutical compositions comprising the GHB salts, as disclosed herein, including before or immediately after the formulations and/or pharmaceutical compositions are ingested or swallowed. This amount can, for example, include the formulations and/or pharmaceutical disclosed herein and any additional aqueous material used to dilute, wash down or chase the formulations and/or pharmaceutical compositions. The additional aqueous material includes for example, water and flavored beverages.

The term, "eating" as used herein, refers to ingesting or consuming calories and/or nutrients by way of solid or liquid food substances.

The term, "cataplexy," as used herein, refers to a condition where a patient exhibits a sudden and transient loss of muscle tone, often triggered by emotions.

The term, "daytime sleepiness," as used herein, refers to a condition where a patient exhibits persistent sleepiness, and often a general lack of energy, even after apparent adequate night time sleep.

The term, "narcolepsy," as used herein, refers to a chronic sleep disorder characterized by excessive sleepiness and sleep attacks at inappropriate times.

The term, "apnea," as used herein, refers to a condition where a patient suspends external breathing.

The term, "sleep time disturbances," as used herein, refers to a condition where a patient exhibits abnormal sleep patterns. Sleep time disturbances can be serious enough to interfere with normal physical, mental and emotional functioning.

The term, "sleep paralysis," as used herein, refers to a condition in which a patient who is falling asleep or awakening form sleep experience an inability to move. It is a transition state between wakefulness and rest characterized by complete muscle weakness.

The term, "hypnagogic hallucination," as used herein, refers to a transition state between wakefulness and sleep where a patient experiences vivid hallucinations.

The term, "sleep arousal," as used herein, refers to a condition where a patient engages in sexual acts while still asleep.

The term, "insomnia," as used herein, refers to a condition where a patient has difficulties falling asleep and maintaining sleep.

The term, "nocturnal myoclonus," as used herein, refers to a condition where a patient has repetitive movement of the limbs during sleep or even wakefulness which is sometimes confused with a seizure.

The term "flavoring" or "flavoring agent," as used herein, refers to a substance that alters the flavor of the composition during oral consumption. A type of "flavoring agent" would be a sweetener.

The term "coloring" or "coloring agent," as used herein, refers to a substance that alters the color of the composition.

The term "bioequivalent", as used herein, describes a formulation and/or pharmaceutical composition that is therapeutically equivalent to a reference product (e.g. Xyrem®) when given under the same conditions in a pharmacokinetic evaluation conforming to FDA Guidance on Bioequivalence Testing; regardless of biopharmaceutical class. A value that is "bioequivalent", as used herein, is meant to refer to a pharmacokinetic value (such as the Cmax or AUC of a formulation described herein) that exhibits substantially similar pharmacokinetic profiles or therapeutic effects. Bioequivalence may be demonstrated through several in vivo and in vitro methods. These methods may include, for example, pharmacokinetic, pharmacodynamic, clinical and in vitro studies. In some embodiments, bioequivalence may be demonstrated using any suitable pharmacokinetic measures or combination of pharmacokinetic measures known in the art, including loading dose, steady-state dose, initial or steady-state concentration of drug, biological half-life, elimination rate, area under the curve (AUC), clearance, the peak blood or plasma concentration (Cmax), time to peak concentration (Tmax), bioavailability and potency. In some embodiments, a value is bioequivalent to a reference pharmacokinetic value when the geometric mean of the AUC and/or the Cmax is between 80% and 125% (e.g., at 90% confidence interval) of the reference pharmacokinetic value.

In some embodiments, a pharmaceutical composition is bioequivalent to a reference pharmaceutical composition when the pharmaceutical composition produces an average Cmax and/or AUC that is substantially the same as the Cmax and/or AUC of the reference pharmaceutical composition when administered under the same conditions. In some embodiments, a pharmaceutical composition is bioequivalent to a reference pharmaceutical composition when the pharmaceutical composition produces a Cmax and/or AUC that is within 80% and 125% of the Cmax and/or AUC of the reference pharmaceutical composition when administered under the same condition. For example, a pharmaceutical composition is bioequivalent to Xyrem® when the pharmaceutical composition produces an average Cmax and/AUC is between 80% and 125% of the Cmax and/or AUC of Xyrem® when administered under the same conditions.

The expression "consists essentially of" as used herein, means that specific further components can be present in a mixture or composition, namely those not materially affecting the essential characteristics of the mixture or composition.

6.2 Pharmaceutical Compositions Comprising Salt Mixtures of GHB

In certain aspects, provided herein are pharmaceutical compositions comprising gamma-hydroxybutyrate (GHB) and one or more pharmaceutically acceptable cations of an alkali metal or an alkaline earth metal. As used herein, "alkali metal" means any of the elements found in Group IA of the periodic table, including, for example, lithium, sodium, and potassium. As used herein, "alkaline earth metal" means any of the elements found in Group II of the periodic table, including, for example, magnesium and calcium.

In certain embodiments, the pharmaceutical compositions comprise GHB and more than one pharmaceutically acceptable cations of an alkali metal or an alkaline earth metal.

In certain embodiments, the pharmaceutical compositions comprise GHB and more than one (two or more) cations selected from the group consisting of Na^+, K^+, Mg^{+2}, and Ca^{+2}. In certain embodiments, the pharmaceutical compositions comprise GHB and all three cations selected from the group consisting of Na^+, K^+, and Ca^{+2}. In certain embodiments, the pharmaceutical compositions comprise less than 100% of the cation Na^+, so as to minimize the amount of sodium, particularly in patients with hypertension, heart disease, renal disease or at risk of stroke or to improve the taste of the compositions. In certain embodiments, the pharmaceutical compositions comprise from about 50% to

about 80% of the cation Na^+. In other embodiments, the pharmaceutical compositions comprise from about 0% to about 40% of the cation Na^+. Each embodiment has a different advantage.

In certain aspects, provided herein are pharmaceutical compositions comprising salts of GHB. As used herein, the term "salt of GHB" or "salts of GHB" is used interchangeably with the term "cation." For example, a pharmaceutical composition comprising GHB and the four cations Na^+, K^+, Mg^{+2}, and Ca^{+2} will be understood by those skilled in the art to also mean a pharmaceutical composition comprising the salts Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. It will be also understood by those skilled in the art that such salts may be in solid form, or may be in partially or fully solvated form, for example, as when dissolved in an aqueous medium. It will be further understood by those skilled in the art, that, depending on the solubility of the salt in the aqueous medium, that the salt may be present in the aqueous medium as solvated cation(s) and anion(s), or as a precipitated solid.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises Na.GHB, and further comprises any one of the salts selected from the group consisting of K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, K.GHB, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB and K.GHB. In certain embodiments, the pharmaceutical composition comprises Na.GHB, K.GHB, and $Mg.(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises Na.GHB and $Mg.(GHB)_2$.

The amounts of the cations below are described in various ranges. The cations can be present in the ranges found in U.S. Pat. Nos. 8,591,922; 8,901,173; and 9,132,107.

In certain embodiments, the Na.GHB salt is present in the mixture in a percentage of at least 50%. In certain embodiments, the Na.GHB salt is present in about 50% to about 80%. In certain embodiments, the Na.GHB salt is present in about 50% to about 70%. In certain embodiments, the Na.GHB salt is present in about 50% to about 60%. In certain embodiments, the Na.GHB salt is present in about 50% to about 55%. In certain embodiments, the Na.GHB salt is present between 40% and 50% and in others between 5% to 45%. In certain embodiments, the Na.GHB salt is present in about 5% to 35%. In certain embodiments, the Na.GHB salt is present in about 5% to 25%. In certain embodiments, the Na.GHB salt is present in about 5% to 10%.

In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 45% and 50% Na.GHB. In certain embodiments, the mixture comprises about 5% to 45% Na.GHB.

In certain embodiments, the mixture comprises at least 50% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 80% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 70% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 60% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 55% Na.GHB. In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 5% to 45% Na.GHB. In certain embodiments, the mixture comprises about 5% to 35% Na.GHB. In certain embodiments, the mixture comprises about 5% to 25% Na.GHB. In certain embodiments, the mixture comprises about 5% to 10% Na.GHB.

In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 45% and 50% Na.GHB. In certain embodiments, the mixture comprises about 5% to 45% Na.GHB.

In certain embodiments, the remaining one, two or three or more cations that are present in the mixture in amounts to make up the remainder of the cations in the formulation and/or pharmaceutical composition. The amount of each depends on the amount of Na^+ and the amount of other cations. For example, if Na^+ is present at 50% and Ca^{+2} and K^+ are also present, then Ca^{+2} and K^+ can each be present in varying amount from 5-40% to add up to the remaining 50%. If Mg^{+2} is also present in the mixture then the non-sodium component 50% is divided three ways. In some embodiments, the mixture does not comprise a significant amount of $Mg.(GHB)_2$ or $Ca.(GHB)_2$, and therefore the formulation and/or pharmaceutical composition does not have a significant amount of $Mg.(GHB)_2$ or $Ca.(GHB)_2$. Care can be taken to adjust any specific cation concentration to levels that are acceptable to patients. It may not be preferred to add any cation to a level that might be disadvantageous to patients generally. For example, potassium has poor tolerability in solution at high doses given on an empty stomach and can also be a problem for patients with kidney impairment.

In certain embodiments, Na^+ is present at 50% and Ca^{2+} and K^+ are also present, then Ca^{2+} and K^+ can each be present in varying amount from 5-45% to add up to the remaining 50%.

In certain embodiments, the K.GHB, $Mg.(GHB)_2$ or $Ca.(GHB)_2$ salt is present in the mixture at about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the K.GHB, $Mg.(GHB)_2$ or the $Ca.(GHB)_2$ salt is absent.

In certain embodiments, the mixture comprises K.GHB, $Mg.(GHB)_2$ or the $Ca.(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% K.GHB. In certain embodiments, the mixture comprises about 0% $Mg.(GHB)_2$. In certain embodiments, the mixture comprises about 0% $Ca.(GHB)_2$.

In certain embodiments, the mixture comprises K.GHB in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%,

about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% K.GHB.

In certain embodiments, the mixture comprises Mg.$(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% Mg.$(GHB)_2$.

In certain embodiments, the mixture comprises Ca.$(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition has reduced sodium compared to Xyrem®, wherein the Na.GHB salt is present in the mixture at about 50% to about 80%.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises at least 50% of a sodium salt of Na.GHB, and further comprises one or more of the following salts, K.GHB, Ca.$(GHB)_2$ and Mg.$(GHB)_2$. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 80%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 70%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 60%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 55%.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more salts of GHB, wherein the mixture comprises of at least 50% of Na.GHB and further comprises one or more of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more salts of GHB, wherein the mixture consists essentially of at least 50% of Na.GHB and one or more of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of three or more salts of GHB.

In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the mixture does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the mixture consists of 50% to 80% Na.GHB, at least 10% K.GHB, and at least 10% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between 50% to 80% Na.GHB, between 30% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between 50% to 80% Na.GHB, between 10% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of between 50% to 80% Na.GHB, between 10% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises about 50% to 80% Na.GHB, about 30% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises about 50% to 80% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of about 50% to 80% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between about 50% to 80% Na.GHB, between about 30% to 40% K.GHB, and between about 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between about 50% to 80% Na.GHB, between about 10% to 40% K.GHB, and between about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of between about 50% to 80% Na.GHB, between about 10% to 40% K.GHB, and between about 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between 50% and 60% Na.GHB, between 20% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between 50% and 60% Na.GHB, between 10% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises about 50% to about 60% Na.GHB, about 20% to about 40% K.GHB, and about 10% to about 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises about 50% to 60% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between about 50% and about 60% Na.GHB, between about 20% and about 40% K.GHB, and between about 10% and about 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between about 50% and about 60% Na.GHB, between about 10% and about 40% K.GHB, and between about 10% and about 20% Ca.$(GHB)_2$.

In certain embodiments the mixture comprises 45% to 55% Na.GHB, 30% to 40% K.GHB, and 10% to 20% Ca.$(GHB)_2$. In certain embodiments the mixture comprises 48% to 52% Na.GHB, 32% to 36% K.GHB, and 14% to 18% Ca.$(GHB)_2$. In certain embodiments, the mixture does not have a substantial amount of Mg.$(GHB)_2$. In other embodiments, the mixture does not have a substantial amount of Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises at least 50% Na.GHB, and further comprises K.GHB and Ca.$(GHB)_2$, In certain embodiments, the mixture comprises between 50% and 60% of Na.GHB, between 10% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$. In certain embodiments, the mixture does not comprise a substantial amount of Mg.$(GHB)_2$. In certain embodiments,

the Na.GHB, K.GHB, and Ca.$(GHB)_2$ salts are present in the mixture in a ratio of about 50%:34%:16%.

In certain embodiments, the pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture comprising between 40% and 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

In certain embodiments, the mixture comprises about 40% to about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the mixture comprises between about 40% and about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture essentially consists of about 40% to about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture which contains between 40% and 50% Na.GHB, wherein the composition is provided to the patient in an aqueous solution of between 25 and 100 mL. In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 40 and 75 mL. In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 55 and 65 mL.

In certain embodiments, the aqueous solution has a volume of about 25 mL to about 100 mL. In certain embodiments, the aqueous solution has a volume of about 40 mL to about 75 mL. In certain embodiments, the aqueous solution has a volume of about 55 mL to about 65 mL. In certain embodiments, the aqueous solution has a volume of about 60 mL.

In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 25 and 75 mL. In certain embodiments, the pharmaceutical composition comprises about 60 mL of an aqueous solution.

In certain embodiments, the pharmaceutical composition comprises between 25 and 100 mL of an aqueous solution. In certain embodiments the pharmaceutical composition comprises between 40 and 75 mL of an aqueous solution. In certain embodiments the pharmaceutical composition comprises between 55 and 65 mL of an aqueous solution.

In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 25 mL to about 100 mL. In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 40 mL to about 75 mL. In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 55 mL to about 65 mL.

In certain embodiments, the pharmaceutical composition is bioequivalent to Xyrem® which is Na.GHB. In certain embodiments, the pharmaceutical composition produces an average maximum GHB plasma concentration (Cmax) that is substantially the same as the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition produces a Cmax that is within 80% and 125% of the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition produces an average maximum GHB plasma area under the curve (AUC) and Cmax that is substantially the same as Na.GHB. In certain embodiments, the pharmaceutical composition produces an AUC that is between 80% and 125% of the AUC of Na.GHB.

In certain embodiments, the pharmaceutical composition is bioequivalent to a pharmaceutical composition comprising about 100% Na.GHB when administered to a patient.

In certain embodiments, the average maximum GHB plasma concentration (Cmax) is within 10% of the Cmax of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, the AUC is within 10% of the AUC of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient.

In certain embodiments, the pharmaceutical composition is formulated as a liquid formulation, wherein the Na.GHB salt is present at less than 40%. In these embodiments, the pharmaceutical composition is more resistant to a food effect and has a lower Cmax compared to Na.GHB.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises less than 40% Na.GHB, and further comprises one or more of the following salts, K.GHB, Ca.$(GHB)_2$ and Mg.$(GHB)_2$. In certain embodiments, the Na.GHB salt is present in the mixture at about 0% to 30%. In certain embodiments, the Na.GHB salt is present in the mixture at about 5% to 25%. In certain embodiments, the Na.GHB salt is present in the mixture at about 5% to 10%.

In certain embodiments, the pharmaceutical composition comprises a mixture of three or more GHB salts, wherein the mixture comprises at least 10% K.GHB, at least 10% Ca.$(GHB)_2$ and at least 10% Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of two or three GHB salts, in addition to Na.GHB, wherein the mixture further comprises 20 to 80%, K.GHB, Ca.$(GHB)_2$ or Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three or more GHB salts, wherein the mixture comprises between 10 and 50% K.GHB, between 10 and 50% Ca.$(GHB)_2$ and between 10 and 50% Mg.$(GHB)_2$ for the non-sodium salts.

In certain embodiments, the Na.GHB, K.GHB, Mg.$(GHB)_2$, and Ca.$(GHB)_2$ salts are present in the mixture at a ratio of about 8%:23%:21%:48%, respectively.

6.2.1 Concentrations and pH Values

In certain embodiments, the pharmaceutical composition comprises an aqueous solution.

In certain embodiments, the concentration of the mixture of salts of GHB in the solution is about 250 mg/mL to about 750 mg/mL, about 350 mg/mL to about 650 mg/mL, about 400 mg/mL to about 600 mg/mL, about 450 mg/mL to about 550 mg/mL. In certain embodiments, the concentration of the mixture of salts of GHB in the solution is centered around 409 mg/mL GHB, which equates to 500 mg/mL Na.GHB. See U.S. Pat. No. 6,472,431, which is incorporated by reference in its entirety.

It will be understood that the maximum solubility of GHB is affected by the pH of the aqueous medium. For example, at about pH 4, the maximum amount of Na.GHB that can be dissolved is about 450 mg/mL. The value of pH that is conducive to GHB solubility increases so that the minimal pH that will dissolve 750 mg/mL GHB was found to be about pH 6.8.

Accordingly, in certain embodiments, the pharmaceutical composition has a pH of about 7.0 to about 9.0, about 7.0 to about 8.5, about 7.3 to about 8.5.

In certain embodiments, the pharmaceutical composition is chemically stable and resistant to microbial growth. In certain embodiments, the pharmaceutical composition is free of preservatives.

It will also be understood that the pH of the aqueous solution affects the resistance of the pharmaceutical composition to microbial growth at about 409 mg/mL GHB, which equates to, e.g., 500 mg/mL Na.GHB. For example, Na.GHB at this concentration (500 mg/mL) is resistant to microbial growth in an aqueous medium when the pH is between about pH 5 and pH 9. Compositions at about pH 6 to about pH 7.5 are particularly resistant to microbial growth. However, at concentrations of GHB greater than about 750 mg/mL above about pH 7.5, the resistance to microbial growth is reduced. See U.S. Pat. No. 6,472,431.

It will be further understood that the chemical stability of GHB is affected by pH. Accordingly, the method for preparing GHB, as described herein, particularly as disclosed in the specific examples, varies with pH. The impurity gamma butyrolactone (GBL) begins to form substantially if the pH is about 6 or less. Compositions with a pH of greater than about 6.0 are preferred to produce chemically stable formulations of GHB. Thus, a preferred range for chemically stable GHB would be from about pH 6 to about pH 9. However, any pH or range of pH values where a clinically acceptable amount of GBL is present is also contemplated as being preferred, and is encompassed by the present invention.

In certain embodiments, a pH adjusting or buffering agent may be added to the composition. The choice of a pH adjusting or buffering agent may affect the resistance to microbial challenge and/or the stability of GHB, as measured by the reduction in assayable GHB. Compositions of GHB, pH adjusted or buffered with malic or other acids are resistant to both microbial growth and chemical degradation of GHB, and are preferred. Other pH adjusting or buffering agents may be selected. Agents that adjust pH that are selected on this basis may undergo a taste testing study. However, any pH adjusting or buffering agent disclosed herein or as would be known to those skilled in the art is contemplated as being useful from the compositions or formulations disclosed herein. Of course, any salt, flavoring agent, excipient, or other pharmaceutically acceptable addition described herein, or as would be known to those skilled in the art, is contemplated as being useful for the compositions or formulations disclosed herein. See U.S. Pat. No. 6,472,431, and Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013, each of which is hereby incorporated by reference in its entirety.

In certain embodiments, the pH adjusting or buffering agent is an acid. In certain embodiments, the pH adjusting or buffering agent is an inorganic acid or an organic acid. In certain embodiments, the pH adjusting or buffering agent is selected from the group consisting of malic acid, citric acid, acetic acid, boric acid, lactic acid, hydrochloric acid, phosphoric acid, sulfuric acid, sulfonic acid, and nitric acid. In certain embodiments, the pH adjusting or buffering agent is malic acid. See U.S. Pat. No. 6,472,431.

6.2.2 Formulations

The aqueous solutions disclosed herein typically comprise an effective amount of GHB, or a salt or mixture of salts of GHB as disclosed herein, which may be dissolved or dispersed in a pharmaceutically acceptable carrier and/or an aqueous medium.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is not appropriate. Supplementary compatible active ingredients can be incorporated into the compositions. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the Food and Drug Administration (FDA). See Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013.

In certain embodiments, the compositions disclosed herein are provided in a formulation, preferably, a liquid formulation, although solid formulations are also contemplated. For any examples of excipients, colorants, flavorants, or other components of the formulation; see Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013.

In certain embodiments, the formulation is chemically stable and resistant to microbial growth. In certain embodiments, the formulation does not need, and may be free of preservatives. In certain embodiments, the level of gamma-butyrolactone (GBL) is 0.1% or less of the formulation. However, if preservatives are added they may include, but are not limited to, xylitol, sodium benzoate, methylparaben, propyl gallate BP, sorbic acid, chlorobutanol, dihydroacetic acid, monothioglycerol, potassium benzoate, propylparaben, benzoic acid, benzalkonium chloride, alcohol, benzoic acid, benzalkonium chloride, benzethonium chloride, benzyl alcohol, butylparaben, cetylpyridinium chloride, ethylenediamine, ethylparaben, ethyl vanillin, glycerin, hypophosphorus acid, methylparaben, phenol, phenylethyl alcohol, phenylmercuric nitrate, propylparaben, sassafras oil, sodium benzoate, sodium propionate, thimerosal and potassium sorbate. Preferred preservatives may be selected from the group comprising, but not limited to, xylitol, sodium benzoate, methylparaben, propylparaben and potassium sorbate. Xylitol is particularly preferred in certain compositions disclosed herein, because it acts as an preservative and a sweetener, is a caries preventative, is less laxative than other sweeteners, and is recommended for diabetics. See U.S. Pat. Nos. 8,324,275 and 8,952,062, and Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013, each of which is incorporated hereby by reference in its entirety.

In certain embodiments, the formulation is suitable for oral administration.

In certain embodiments, the formulation additionally comprises a flavoring agent. Preferred sweeteners or flavoring agents would be microbially non-metabolizable. Especially preferred sweeteners or flavoring agents would be carbohydrates such as xylitol and sorbitol. Such flavoring agents include, but are not limited to, acacia syrup, anethole, anise oil, aromatic elixir, benzaldehyde, benzaldehyde elixir-compound, caraway, caraway oil, cardamom oil, cardamom seed, cardamom spirit, cardamom tincture-compound, cherry juice, cherry syrup, cinnamon, cinnamon oil, cinnamon water, citric acid, citric acid syrup, clove oil, coca, coca syrup, coriander oil, dextrose, eriodictyon, eriodictyon fluidextract, eriodictyon syrup-aromatic, ethyl acetate, ethyl, vanillin, fennel oil, ginger, ginger fluidextract, ginger oleoresin, glucose, glycerin, glycyrrhiza, glycyrrhiza elixir, glycyrrhiza extract, glycyrrhiza extract-pure, glycyrrhiza fluidextract, glycyrrhiza syrup, honey, non-alcoholic elixir, lavender oil, citrus extract or oil, lemon oil, lemon tincture, mannitol, methyl salicylate, nutmeg oil, orange-bitter-elixir,

orange-bitter-oil, orange flower oil, orange flower water, orange oil, orange peel-bitter, orange-peel-sweet-tincture, orange spirit-compound, compound, orange syrup, peppermint, peppermint oil, peppermint spirit, peppermint water, phenylethyl alcohol, raspberry juice, raspberry syrup, rosemary oil, rose oil, rose water, saccharin, saccharin calcium, saccharin sodium, sarsaparilla syrup, sorbitol solution, spearmint, spearmint oil, sucralose, sucrose, syrup, thyme oil, tolu balsam, tolu balsam syrup, vanilla, vanilla tincture, vanillin or wild cherry syrup.

In certain embodiments, the formulation additionally comprises a coloring agent. Preferred coloring agents would be microbially non-metabolizable.

In certain embodiments, the formulation is administered in a single or multiple dosage regimen.

Any of the above formulations may be prepared and/or packaged as a powdered or dry form for mixing with an aqueous medium before oral administration, or they may be prepared in an aqueous medium and packaged. After mixing with an aqueous medium, preferably to prepare a solution, these formulations are resistant to both microbial growth and chemical conversion of GHB to GBL, thereby increasing the shelf-life of therapeutic formulations of GHB, or salt or mixture of salts of GHB, in an aqueous medium. These formulations then provide an easily titratable liquid medium for measuring the dosage of GHB, or salt or mixture of salts of GHB, to be administered to a patient. Additional embodiments of the composition and methods of preparation are described below and in the examples.

In certain embodiments, especially with Na.GHB amounts between 40% and 50%, the formulation is present in a low volume of aqueous solution. As described herein, by "low volume" it is meant to include an aqueous solution of about 100 mL or less, including the aqueous medium and any wash or chase volume, for administration of a single GHB dose. Preferably the low volume is between about 25 mL to 75 mL, or between 55 mL to 65 mL of total aqueous volume given to the patient. In certain embodiments, for example, formulations with reduced sodium, the formulation requires less aqueous volume in order to be ingested, is more palatable, provides better patient compliance, is more tolerable, and/or is bioequivalent in comparison to GHB formulations of Na.GHB. It should be understood by those skilled in these arts that 25-100 mL (or about 1-3 ounces) of fluid is an acceptable amount of aqueous solvent to dilute the formulations disclosed herein, in order to ingest, improve taste, and/or "wash down" the GHB salts. For certain individuals, having a reduced-volume for administration offers an improved nightly dosing regimen which may alleviate unwanted side-effects associated with consuming liquids before bedtime, such as bed-wetting, restlessness and/or other sleep time disturbances.

The GHB, or salt or mixture of salts of GHB disclosed herein, may be lyophilized for more ready formulation into a desired vehicle or medium where appropriate. The GHB or salt(s) thereof may also be formulated for parenteral administration, e.g., formulated for injection via intravenous, intraarterial, intramuscular, sub-cutaneous, intralesional, intraperitoneal or other parenteral routes. The preparation of a pharmaceutical composition that comprises an aqueous solution that contains GHB or salt(s) thereof as an active component or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including, e.g., aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions of the active compounds as free acid or pharmacologically acceptable salts can be prepared in water suitably mixed with hydroxypropyl cellulose and/or a pharmaceutically acceptable surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof as well as in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to further prevent the growth of microorganisms.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, or the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a substance, such as lecithin (e.g., a coating), by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by any of the preservatives described herein, or as would be known to those skilled in the art, including various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with, various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, or highly, concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent (although DMSO may not now be a permitted human drug) is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solu-

tions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of fluid or injected at the proposed site of infusion, (see, e.g., "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The GHB may be prepared in a formulation and/or pharmaceutical composition disclosed herein to comprise about 100 to about 10,000 milligrams per dose as administered to the patient. The typical dose range is approximately 4.5-9 g/day; see the Xyrem® Product Insert. Other dose ranges include 6-8 g/day multiple or single doses can be administered but it is typical to give two divided doses per day. The Xyrem® instructions recommend two equally divided doses.

In addition to the pharmaceutical compositions formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g., tablets or other solids; liposomal formulations; time release capsules, such as sustained or delayed release forms, including beads, pellets, or resins; and any other form currently used, including creams, which then may be admixed with an aqueous medium for oral administration.

One may also use nasal solutions or sprays, aerosols or inhalants in connection with the pharmaceutical compositions and/or formulations disclosed herein. Nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, the aqueous nasal solutions usually are isotonic and slightly buffered to maintain a pH of 5.5 to 6.5, though other pH ranges disclosed herein the specific examples, such as pH 3 to about pH 9, or pH 6 to about 7.5, are contemplated. In addition, preservatives, similar to those used in ophthalmic preparations, and appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known and include, for example, antibiotics and antihistamines and are used for asthma prophylaxis.

The preferred oral formulations may include such normally employed excipients, as, for example, pharmaceutical grades of xylitol, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders to be admixed with an aqueous medium. In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent or assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or the GHB or salt(s) thereof may be packaged separately from or in combination with the excipients, salts, flavorings or any other components described herein, to be admixed with an aqueous medium for oral or injectable formulations, or they may be incorporated directly with the food (i.e. a beverage) of the diet.

For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of tablets, buccal tablets or tabs, troches, capsules, elixirs, suspensions, syrups, wafers, and the like, to be admixed with an aqueous medium. Such compositions and preparations should contain at least 0.1% of the active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, natural as gum tragacanth, acacia, cornstarch, or gelatin or synthetic as polyvinyl acetate; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a natural or synthetic flavoring agent. When the dosage unit form is a capsule for admixing with a specific volume of an aqueous medium, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with sugar, natural or synthetic polymers, or both. A syrup or elixir may contain the active compounds, sucrose as a sweetening agent, a preservative, a dye and/or a flavoring.

One embodiment of the formulations disclosed herein can be a solid with different release properties. One embodiment is a unit dosage form that is a tablet for immediate release comprising a relatively high weight-percentage of sodium oxybate, in combination with a relatively small weight-percentage of total excipients. This permits the tablets to contain/deliver a pharmaceutically effective amount of sodium oxybate in each tablet with a delivery profile similar to that of the liquid form. The tablets are bioequivalent to the liquid form. See U.S. Pat. Nos. 8,771,735 and 8,778,398. Other embodiments provide controlled release dosage forms for delivery of GHB or salt(s) thereof. The controlled release dosage forms may incorporate both controlled release and immediate release formulations in a single unit dosage form. See U.S. Publication No. 2012/0076865. Another embodiment includes the use of both immediate release and controlled release forms mixed together or one after the other. In one embodiment the immediate release portion could be between 10-50%, or 20-30% and the controlled release portion comprising the remaining amount. In some embodiments the amounts of the different salts can be different in each of the immediate or controlled release portions.

Additionally, any excipient, salt, acid, pH-mediating, adjusting or buffering compound or agent, flavoring, solution, solvent, dispersion, glycerol, glycol, oil, antibacterial and antifungal agents, antibiotics and antihistamines, binders, disintegrating agents, lubricants, sweetening agents, or any other additive or ingredient from those enumerated above or in the examples, or in any pharmaceutically acceptable composition or carrier described herein, or as would be known by one of skill in the art, is contemplated for use in aqueous mediums or solid forms of the pharmaceutical compositions disclosed herein. One or more of these compositions may be packaged with GHB or salt(s) thereof, or packaged separately from GHB or salt(s) thereof prior to consumption. If packaged separately, useful pharmaceutical compositions may be obtained by mixing GHB or salt(s) thereof with the other components with an aqueous medium prior to consumption. Such components may be packaged in a kit, described below.

Also provided herein are therapeutic kits comprising GHB, or a salt or mixture of salts of GHB, as disclosed herein. Such kits will generally contain, in suitable container, a pharmaceutically acceptable formulation of the GHB or salt(s) thereof. The kit may have a single container, or it may have distinct container for each component, or distinct container for various combinations of components.

When the components of the kit are provided in one or more liquid formulations, the liquid formulation is an aqueous medium, with a sterile aqueous solution being particularly preferred. The pharmaceutical compositions may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, vial, ampule or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, or even applied to and mixed with the other components of the kit.

However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, pouch syringe or other container means, into which the formulation or components thereof are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

The kits will also typically include a means for containing the vials in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained.

In certain embodiments, the kits contain one or more bottles of liquid formulation comprising GHB or salt(s) thereof, two dosing cups with child-resistant caps, a liquid measuring device and a medication guide.

In certain embodiments, the kits contain two different GHB formulations in separate bottles. In certain embodiments, the kits contain two bottles of liquid formulation comprising GHB or salt(s) thereof, wherein two different formulations are provided in at least two separate bottles. In certain embodiments, the kits contain two or more bottles of liquid formulation comprising GHB or salt(s) thereof, wherein two different formulations are provided in at least two separate bottles, and wherein also provided are two dosing cups with child-resistant caps, one or more liquid measuring device and a medication guide. Preferably, the two different formulations are a first-dose formulation comprising an aqueous solution, the aqueous solution is a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and the second-dose formulation comprising an aqueous solution comprising from 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

Irrespective of the number or type of containers, the kits may also comprise, or be packaged with, an instrument for assisting with the injection/administration or placement of the pharmaceutical composition within the body of an animal. Such an instrument may be a drinking cup, syringe, pipette, or any such medically approved delivery vehicle. Where two more formulations are provided in the kit, optionally, one or more of the instruments or formulations can be color-matched or labeled to indicate which of the two doses are contained within it. Furthermore, the drug product containers can be differentiated by color, shape or other identifying features. The containers can be bound together (for example, by shrink wrapping) or assembled into the kit in such a way to minimize misplacement or discourage dispensing of one product for both dosings. Where two or more formulations are provided as granules or other rapidly dissolving dosage form, twin sachets with a perforated divider can facilitate dose preparation. These could be labeled, for example, as "1st dose" and "2nd dose".

Furthermore and to distinguish between prepared formulations prior to administration, one or both of the formulations can include a flavorant, odorant, or colorant to render it substantially different from the other. The additive may also be provided separately in the kit so that it can be added to the water either immediately before or after dispensing each formulation. Also, the administration devices for each dose may be distinguished based on a number of features such as color, shape, etc. so that that patient can easily administer each dose.

6.2.3 Methods of Treatment

All the pharmaceutical compositions and formulations provided herein can be used in all the methods provided herein. For example, the pharmaceutical compositions and formulations provided herein can be used in all the methods for treating all diseases, disorders or conditions provided herein. Thus, the pharmaceutical compositions and formulations provided herein are for use as a medicament. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating a disease or condition in a subject that is suitable to treatment by GHB, comprising administering a pharmaceutical composition or formulation disclosed herein.

The pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, are also contemplated to be useful in the treatment of any of these disorders or conditions in patients. GHB has also been used alone as a narcotic in patients with terminal cancer. GHB has been used with other analgesics, neuroleptics, or with a subliminal barbiturate dose for use as an anesthesia. It is also contemplated that the pharmaceutical compositions and formulations disclosed herein may be used as a narcotic, hypnotic, or as a soporific. It is further contemplated that the pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, may be used in combination with analgesics, neuroleptics or barbiturates for use as an anesthesia. See the methods described at the end of U.S. Pat. No. 6,472,431.

The pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, may be prepared and administered by any of the means described herein, particularly those described in the section "Formulations" and the examples, or by any means as would be known to those of skill in the art.

Accordingly, in certain aspects, are methods of treatment comprising administration to a patient of the pharmaceutical compositions or formulations comprising mixed salts GHB disclosed herein.

In certain embodiments, the pharmaceutical compositions or formulations comprising mixed salts of GHB, disclosed herein, are useful in the treatment of cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy.

In certain embodiments, the pharmaceutical compositions or formulations comprising mixed salts of GHB, disclosed herein, are useful in the treatment of conditions responsive to GHB, for example, sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

Accordingly, in certain embodiments, provided herein is a method for treating a disease or condition in a subject that is suitable to treatment by GHB, comprising administering a pharmaceutical composition or formulation disclosed herein.

In certain embodiments, also provided herein is a method of treating a disease or condition that is suitable for treatment with GHB wherein the method comprises administering to a patient a pharmaceutical composition comprising from 50% to about 80% of Na.GHB, wherein the pharmaceutical composition is in an oral dosage form and wherein administration of the pharmaceutical composition produces a GHB Cmax which is bioequivalent to the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In preferred embodiments, the disease is cataplexy and/or narcolepsy. In certain embodiments, the disease or condition is selected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In certain embodiments, the mixture of salts which from about 50% to about 80% of Na.GHB further comprises one or more salts selected from the group consisting of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, also provided herein is a method of treating a disease or condition that is suitable for treatment with GHB wherein the method comprises administering to a patient a pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture comprising between 40% and 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the disease is cataplexy and/or narcolepsy.

In certain embodiments, when administered to a patient, the pharmaceutical composition produces a GHB Cmax which is within 10% of the Cmax of Na.GHB. In certain embodiments, the Cmax is within 10% of the Cmax of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, when administered to a patient, the pharmaceutical composition produces a GHB Cmax that is bioequivalent to the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition is bioequivalent to a pharmaceutical composition comprising about 100% Na.GHB when administered to a patient. In certain embodiments, the AUC is within 10% of the AUC of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In preferred embodiments, the disease is cataplexy and/or narcolepsy. In certain embodiments, the disease or condition is selected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In certain embodiments, the methods of treatment comprising administration of the pharmaceutical compositions or formulations comprising mixed salts GHB disclosed herein.

In certain embodiments, the method comprises oral administration of the pharmaceutical compositions or formulations comprising mixed salts GHB, disclosed herein, in a multiple dosage regimen.

In certain embodiments, the multiple dosage regimen comprises one or more steps, as follows: (i) diluting an aqueous solution comprising about 409 mg/mL of gamma-hydroxybutyrate (GHB) with an aqueous medium to provide a first dose of the mixture of salts; (ii) diluting an aqueous solution comprising about 409 mg/mL of GHB with an aqueous medium to provide a second dose of the mixture of salts; (iii) orally administering to a patient having narcolepsy the first dose; and (iv) orally administering to the patient having narcolepsy the second dose within 2.5 to 4 hours following the first dose. The first and/or second doses can be administered according to the instructions on the label as appropriate.

In certain embodiments, two nightly doses of GHB or a salt there are administered to the patient.

In certain embodiments, the first dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution of a mixture of two or more GHB salts, the mixture comprising less than 40% Na.GHB, and further comprising one, two, three or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the first dose is administered within 4 hours of eating and produces a GHB Cmax which is less than the Cmax of Na.GHB; and the second dose of GHB salts is a pharmaceutical composition of GHB comprising a mixture of two or more GHB salts, the mixture comprising at least 50% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the second dose produces a GHB Cmax which is substantially equivalent to the Cmax of Na.GHB. In certain embodiments, the multiple dosage regimen comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising 0% to 40% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, with an aqueous medium to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80%

of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering the first dose to a patient suitable for treatment with GHB; and (iv) orally administering the second dose to the patient within 2.5 to 4 hours following the first dose. In preferred embodiments, the patient is suitable for treatment with GHB has cataplexy or narcolepsy.

In certain embodiments, the first dose comprises a pharmaceutical composition comprising less than 40% Na.GHB and at least two other GHB salts selected from the group of K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the first dose is administered within 4 hours of eating. In certain embodiments, the mixture further comprises two or more salts selected from the group consisting of K.GHB, Ca.$(GHB)_2$, and Mg. $(GHB)_2$.

In certain embodiments, the disease or condition is selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder, an endocrine disturbance, hypoxia or anoxia of tissues, and an increased level of intracranial pressure.

In certain embodiments, the first dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution of less than 100 mL, the aqueous solution comprises a mixture of three GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from between 10-60% K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the first dose is administered within 4 hours of eating and produces a GHB Cmax which is less than the Cmax of Na.GHB.

In certain embodiments, the second dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution, the aqueous solution comprising from 50% to about 80% of Na.GHB, and from between 10-60% K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein administration of the second dose produces a GHB Cmax which is substantially bioequivalent to the Cmax of Na.GHB. In certain embodiments, the second dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution which comprises a mixture from 50% to about 80% of Na.GHB, and wherein administration of the second dose produces a GHB Cmax which is substantially bioequivalent to a composition comprising Na.GHB.

In certain embodiments, 4.5 and 9 grams/day are administered to the patient in two divided doses.

In certain embodiments, 6 and 8 grams/day are administered to the patient in two divided doses.

In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure.

It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including: the metabolic stability and length of action, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

6.2.4 Methods of Making

In certain aspects, provided herein are some exemplary methods of making the compositions or formulations comprising mixed salts GHB disclosed herein. Several different methods of making have been reported in the literature (see, e.g., U.S. Pat. Nos. 4,393,236; 4,983,632; 6,472,431; 8,461,203; 8,591,922; 8,901,173; and 9,132,107; and U.S. Publication No. 2016/0058720, each of which is incorporated by reference in its entirety; see also Ferris and Went, 2012, *Forensic Science International* 216: 158-162). Those skilled in the art will recognize that these methods can be incorporated in the making of the compositions or formulations comprising mixed salts GHB disclosed herein. Other methods will be known to those of skill in the art.

In certain embodiments, mixtures of GHB salts can be made by direct reaction of GBL with an aqueous mixture of one of more of the following bases: sodium hydroxide, potassium hydroxide, calcium hydroxide, and magnesium hydroxide. After reaction the mixture may then be filtered under mild vacuum.

In certain embodiments, a solvent, such as water, is used to dissolve the GHB salt mixture to a desired concentration, for example, by adjusting the amount of water in the mixture.

In certain embodiments, the concentration of a GHB salt solution is adjusted by concentrating the mixture using standard methods, such as evaporators, reverse osmosis, and similar techniques known to those skilled in the art.

In certain embodiments, the method of making comprises reacting gamma-butyrolactone (GBL) with one or more bases selected from the group consisting of sodium hydroxide, potassium hydroxide, magnesium hydroxide, and calcium hydroxide.

In other embodiments, the method of making comprises, for example, reacting GBL with one or more of sodium carbonate, potassium carbonate, or magnesium carbonate to provide the sodium, potassium, and magnesium oxybate (Na.GHB, K.GHB, and Mg.$(GHB)_2$) mixture. Such embodiments are particularly suitable to avoid precipitation of calcium carbonate when carbonate salts of sodium, potassium, and/or magnesium are employed.

In still other embodiments, a solution of calcium oxybate can be transformed to a mixture of oxybate salts by exchanging with a mixture of cation exchange resins loaded with the desired cations. Alternatively, a solution of calcium oxybate can be transformed to a mixture of oxybate salts by precipitation with a mixture of acid salts of other cations when the calcium salt is practically insoluble. After filtration or other means of removing the precipitated calcium salt or the exchanged cation exchange resin, the mixed oxybate salt solution is obtained.

In other embodiments, a mixture of cations associated with oxybate may include a proton. This can be achieved in similar fashion as cation exchange or displacement precipitation described above, with the exception that a H-form cation exchange resin or the free acid or partially neutralized salt of the precipitating anion is employed, respectively. Ideally to promote chemical stability, such embodiments should be produced in solid form and suspended or dissolved in water upon administration. In yet another embodiment, the undissolved solid (exchanged cationic resin or precipitated salt) can be ingested with the dose provided neither dissolves appreciably in the GI tract.

In certain embodiments, the reaction is carried out in a single vessel. For example, a mixture of Na.GHB, K.GHB, Mg.$(GHB)_2$, and Ca.$(GHB)_2$ may be made by direct addition of GBL to in a single vessel containing an aqueous mixture of sodium hydroxide, potassium hydroxide, magnesium hydroxide, and calcium hydroxide.

In certain embodiments, the reaction is carried out in multiple vessels and the product is subsequently combined. For example, Ca.$(GHB)_2$ may be made by direct addition of GBL to aqueous sodium hydroxide, and the product combined with Mg.$(GHB)_2$.

In certain embodiments, the methods of making include methods of making the pharmaceutical compositions and formulations disclosed herein.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the and scope of the invention.

7. EXAMPLES

Example 1: Synthesis of Mixed Oxybate Solutions

The following synthetic examples provide exemplary syntheses of mixture of oxybate salts. Alternate methods of synthesizing mixtures of oxybate salts, including methods of synthesizing additional salts of oxybate are described below; still other alternate synthetic methods will be apparent to those skilled in the art. See also U.S. Pat. Nos. 8,461,203; 8,591,922; 8,901,173; and 9,132,107; and U.S. Publication No. 2016/0058720; each of which is incorporated by reference in its entirety.

Mixed oxybate salt solutions can be made conveniently by at least two methods. When multiple different formulations are desired, one of skill in the art can mix solutions of individual salts having the same molar oxybate concentration to arrive at the desired cation blend. On the other hand, for commercial implementation or single-batch manufacturing one can perform a one-pot reaction with GBL and the two or more bases in the desired cationic proportions. Both methods are described below.

Example calculations of molar equivalents and % wt/wt for salt mixtures are also shown below Table 1.

TABLE 1

Example Calculations

Base	Base MW	Purity	Grams Amount	Base mMols	Stoich. Ratio	Base mEQ	% molar equiv GHB	Salt	Salt MW	Salt mass grams	Salt wt/wt %	Conc mg/mL
NaOH	40.00	98.50%	1.398	34.43	1	34.43	8.5%	Na•GHB	126.09	4.34	8.5%	42.61
KOH	56.11	86.72%	7.337	113.40	1	113.40	28.0%	K•GHB	142.20	16.12	31.4%	158.29
$Ca(OH)_2$	74.10	99.00%	6.268	83.74	2	167.49	41.4%	$Ca•(GHB)_2$	246.27	20.62	40.2%	202.46
$Mg(OH)_2$	58.32	99.50%	2.611	44.55	2	89.09	22.0%	$Mg•(GHB)_2$	230.50	10.27	20.0%	100.80
Total			17.614	276.11		404.40	100.0%			51.36	100.0%	504.17

Base	Each of four bases used in this example
Base MW	Molecular weight of the base
Purity	Purity provided by manufacturer. It is assumed that impurities are non-reactive.
Gram Amount	Amount, in grams, of each base charged to the reaction
Base mMols	Corresponding amount, in millimoles, of pure base (that is, Purity × Gram-Amount × 1000/Base-MW)
Stoichiometry Ratio	The number of GHB moles reacted with each mole of base
Base mEQ	Base equivalents for reaction with GHB (that is, Base-mMols × Stiochiometry-Ratio). This is also the Oxybate or GHB equivalents value.
% molar equiv GHB	Molar composition of salts expressed as Percent of Oxybate Equivalents
Salt	The oxybate salt species
Salt MW	Molecular weight of the oxybate salt
Salt-mass-grams	Mass of salt produced by reaction (that is, Base-mMols × Salt-MW/1000)
Salt wt/wt %	Normalized weight percent
Conc. (mg/ml)	Concentration in mg/ml equivalent to a 3.97M Na-GHB solution (500 mg/ml sodium oxybate). That is, 3.97 × (% equiv-GHB) × (Salt-MW)/(Stoich. Ratio)

Example 1.1: Manufacturing Mixed Salts Solutions

Four individual oxybate salt solutions at equal oxybate strength (409 mg/mL) were made as follows:

Magnesium oxybate (Mg.$(GHB)_2$) solution was made by combining 124.6 g water and 20.36 g magnesium hydroxide in a magnetically-stirred 250 mL square glass bottle. 58.04 g of GBL was then added to the base suspension and then heated up to 80° C. with stirring. After 4 hours, a pH verification indicated completion of reaction (pH 8.5). Water was added to compensate for evaporation. The reaction mixture was then centrifuged, and supernatant filtered through 0.45µ. PVDF Stericup under vacuum. The pH of filtrate was 8.1. Yield: 177.4 g solution. Assay (HPLC-UV): 100.1%

Potassium oxybate (K.GHB) solution was made by adding 60.10 g potassium hydroxide to 144.01 g water in a magnetically-stirred 250 mL square glass bottle. After complete dissolution, 78.52 g GBL was weighed into a separate glass beaker. Approximately half the GBL was added initially with instant reaction, and then the solution was cooled in ice water to approximately 30° C. The remainder of the GBL was then added with stirring, and the solution maintained at 60° C. for 2.5 hours. The pH was 13.5. The pH was then adjusted to 8.1 by adding 10% HCl solution. Water was added to restore the initial reaction mass. The solution was then filtered through 0.45µ. PVDF Stericup under vacuum. Yield: 281.8 g solution. Assay (HPLC-UV): 98.6%.

Calcium oxybate (Ca.$(GHB)_2$) solution was made by combining 210.5 g water and 45.41 g calcium hydroxide in a magnetically stirred 500 mL square glass bottle. Next, 102.41 g GBL was added slowly while stirring, and then the reaction was maintained at 80° C. on a temperature-controlled hotplate (surface set point 183° C.). After 2 hours, the

mixture was cooled and water was added to compensate for evaporation. The solution was centrifuged, and supernatant was then filtered through 0.45μ. PVDF Stericup under vacuum. The initial pH of filtrate was 10.5, and was adjusted to 7.9 by addition of 10% HCl solution. Yield: 328.6 g solution. Assay (HPLC-UV): 99.0%

Sodium oxybate (Na.GHB) solution was made by adding 46.6 g sodium hydroxide to 200.1 g water in a magnetically stirred 500 mL square glass bottle. 99.00 g GBL was weighed into a separate beaker. After complete dissolution of the sodium hydroxide, about half of the GBL was added to the reaction mixture causing it to heat. After cooling to about 30° C. in ice water, the remaining GBL was added and then allowed to react with stirring on a hotplate at 60° C. for 2 hours. The pH after reaction was 12.36, and was adjusted to 8.13 by addition of 10% HCl solution. Water was added to restore the initial reaction mass. The solution was then filtered through a 0.45μ. PVDF Stericup under vacuum. Yield: 340.3 g. Assay (HPLC-UV): 100.6%.

For each desired oxybate salt mixture below, the individual solutions were blended volumetrically with an oral dosing syringe into a 250 mL glass beaker with stirring. The blend order, where applicable, was sodium, potassium, calcium, and then magnesium oxybate. 178 mg of sucralose was then added and dissolved. The target cation blends (in equivalents) and volumes of individual solutions used are shown in Table 2 below.

TABLE 2

Target Cation Blends and Volumes of Exemplary Solutions

	% equivalents				Volume (mL) of oxybate solution (#1-#4 above) for total batch 150 mL				Assay
Solution	Na	K	Ca	Mg	Na (#4)	K (#2)	Ca (#3)	Mg (#1)	% Label
507-A	33	34	33	0	49.5	51.0	49.5	0	98.9
507-G	23.3	19.2	40	17.5	35.0	28.8	60.0	26.3	99.2
507-C	33	0	48	19	49.5	0	72.0	28.5	100.0
507-D	50	34	16	0	75.0	51.0	24.0	0	98.8

Example 1.2: Direct, One-Pot Reaction Method to Achieve Various Mixtures

To achieve any combination of oxybate salts, the stoichiometry calculations are adjusted to reflect (a) the strength of individual bases and (b) the use of an excess for the weakest base (calcium or magnesium). The strength of bases used in the Example above were 99.7% (NaOH), 86.0% (KOH), 99.0% ($Ca.(OH)_2$), and 98.5% ($Mg.(OH)_2$). A 1% excess is applied as the weakest divalent base present (calcium or magnesium, in that order of precedence). A larger or smaller excess may be warranted, depending on the level of confidence in the assay values or repeatability of dispensing to the process. A larger excess will increase confidence in completing the reaction, but incur more filtration load. A smaller excess threatens to inadequately complete the reaction, resulting in higher than desired GBL levels.

To make 150 mL batches roughly equivalent in composition to those of Example 1.1, the stoichiometry is as shown in Table 3 below.

TABLE 3

Stoichiometry of Bases used for Exemplary Solutions

	grams base required				Excess		GBL	Water	Total
Solution	NaOH	KOH	$Ca(OH)_2$	$Mg(OH)_2$	As base	grams	grams	grams	grams
507-A	7.88	13.21	7.35	0.00	$Ca(OH)_2$	0.22	51.27	98.56	178.5
507-G	5.57	7.46	8.91	3.12	$Mg(OH)_2$	0.17	51.27	102.00	178.5
507-C	7.88	0.00	10.70	3.38	$Mg(OH)_2$	0.17	51.27	105.09	178.5
507-D	11.95	13.21	3.57	0.00	$Ca(OH)_2$	0.22	51.27	98.29	178.5

The water is weighed into a tared 250 mL beaker with spinbar. Next, bases are weighed and added in order of sodium, potassium, calcium, and magnesium as applicable. After sodium or potassium hydroxide is added, the mixture is stirred until complete dissolution is observed. The required excess is added at the same time as the respective base is charged. Next, 51.27 g of GBL is added slowly while monitoring temperature and with stirring. If the temperature exceeds about 80° C., then GBL addition is slowed until the temperature cools to about 60° C. After GBL addition is complete, the setup is moved to a 60° C. environmental chamber to complete the reaction. (Alternatively, a temperature-controlled hotplate can be employed.) Sodium and potassium hydroxide react almost instantly with GBL. $Ca.(OH)_2$ requires about 1 h to react at 60° C., and $Mg.(OH)_2$ requires about 3 h at 80° C. or overnight (12 h) at 60° C. Therefore, mixtures lacking $Mg.(OH)_2$ (507-A and 507-D) are held at 60° C. for about 1 h. Mixtures 507-G and 507-C are held at 60° C. overnight or 80° C. for 3 h.

After reaction, water is added to compensate for evaporation and restore the original reaction mass (178.5 g net). The reaction mixtures are then centrifuged followed by vacuum filtration through a 0.45μ. PVDF Stericup. Finally, the pH is adjusted with 10% HCl solution, as needed, to a value of 8.0. For mixtures containing magnesium, no adjustment is required if the pH is below 9. Finally, 0.18 g of sucralose is added and dissolved into the solution.

Example 2: Pharmacokinetic Testing of Formulations

This Example provides protocols and results for bioequivalence testing of the formulations disclosed herein. Four sets of bioequivalence testing were performed with various mixed salt formulations compared with Xyrem® as the reference. Unless stated otherwise, this and subsequent examples have oxybate salt concentrations stated in a "molar equivalent percent" basis. Furthermore, in the tables and figures where applicable:

- "Treatment" refers to the formulation and the dosing regimen (fed or fasted), for which various formulations were tested at a dose equivalent to 4.5 g sodium oxybate.
- "N" refers to the number of subjects for which evaluable results were obtained
- "Vol" refers to the volume of administration (mL) given with the 9 mL dose of drug product
- "Cmax" refers to the average of the maximum plasma concentration (in oxybate mg/L or ug/mL) achieved in individual patients
- "Cmax Ratio" refers to the ratio of Cmax value compared to that of fasted state Xyrem® and expressed as a percentage
- "AUC" refers to the area under the curve of plasma vs time, either the last time point where the concentration was above the limit of quantitation or projected out to infinite time and expressed in units of h*mg/L.
- "AUC ratio" refers to the ratio of AUC to that of fasted state Xyrem® and expressed as percentage
- "Na", "K", "Ca", and "Mg" refer to the cation content of the formulation given, in Molar Equivalent %, of sodium, potassium, calcium, and magnesium, respectively.

Example 2.1: Testing of Formulation "O"

Formulation "O" was manufactured as (equivalent %) 8% sodium, 23% potassium, 48% calcium, and 21% magnesium oxybate at 409 mg/mL mixed salt concentration or 409 mg/mL oxybate. The four bases were suspended or dissolved in water, then gamma butyrolactone was added and the reaction mixture was held at 80° C. for about 3 hours. Subsequently, mixture was cooled and then depth filtered, carbon filtered, and then flowed through a polishing filter. Finally, sucralose was added to a level of 0.1% w/v in the final solution.

Formulation "O" was tested for bioequivalence relative to Xyrem® (Formulation "X", commercial sodium oxybate solution of the same molar concentration and comparable pH as "O") and in the fasted as well as fed state. The study was compliant with the FDA guidance for food effect studies ("Guidance for Industry: Food-Effect bioavailability and Fed Bioequivalence Studies", FDA December 2002), incorporated herein by reference in its entirety. In both fasted and fed treatments, the Guidance indicates that the drug product should be administered with 240 mL of water. Thirty-six patients were recruited and 34 patients completed successfully. The results are shown in FIG. **1** and in Table 4 below.

TABLE 4

	Conditions and Results in Study 13-010 Using 240 mL Liquid Volume									
	Number	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	of Patients	(mL)	(mg/L)	ratio	(mg•h/L)	ratio	Na	K	Ca	Mg
O, fasted	34	240	102.3	76%	238.7	89%	8	23	48	21
O, fed	36	240	77.7	58%	216.0	81%	8	23	48	21
X, fasted	32	240	134.6	100%	268.1	100%	100	0	0	0
X, fed	36	240	84.9	63%	233.0	87%	100	0	0	0

Example 2.2: Testing of Blends of Xyrem® and Formulation "O"

As an extension to the study described in Example 2.1, the same formulation "O" and Xyrem® reference were tested in two different proportions to determine whether bioequivalence could be achieved with the same proportion of the three non-sodium cations but with higher sodium content. New patients were recruited for the single dose crossover study, but the study was otherwise done in a manner comparable to Example 2.1 except fewer patients were evaluated. The results are shown in FIG. **2** and Table 5 as expressed in mean values. Bioequivalence was not achieved even at 49% sodium (the confidence interval for that formulation was between 73.8-97.5%).

TABLE 5

	Conditions and Results in Study 13-010 Part 2 using 240 mL Liquid Volume									
	Number	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	of Patients	(mL)	(mg/L)	ratio	(mg•h/L)	ratio	Na	K	Ca	Mg
2.5 g O + 2.0 g X, fasted	21	240	109.4	84%	241.3	96%	49	13	27	12
3.75 g O + 0.75 g X, fasted	19	240	98.18	75%	228.4	91%	23	19	40	18
X, fasted	17	240	130.2	100%	251.4	100%	100			

Example 2.3: Testing of Alternative Cationic Blends

To test for negative effects of certain cations and also to investigate other four-cation blends, the formulations of Example 1.1 were tested in a crossover fasted state bioequivalence study involving 35 patients. In contrast to the preceding two examples, the volume of administration was reduced to 60 mL. The results are shown in FIG. **3** and Table 6.

Surprisingly, as shown in FIG. **3** and Table 6, Formulation 507-D with 50% sodium met the bioequivalence criteria, as it had a Cmax ratio of 92% and nearly identical average plasma profile compared to Xyrem®. In contrast, Formulations 507-A and 507-C, both with 33% sodium but differing by exclusion of either potassium or magnesium, had nearly identical and lower Cmax values (78% and 76%, respectively), and therefore did not meet the bioequivalence criteria.

TABLE 6

Conditions and Resu ts in Study 15-008 using 60 mL Liquid Volume, n = 35 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	(mg•h/L)	ratio	Na	K	Ca	Mg
507-A, fasted (no Mg)	60	102.2	77%	241	85%	33	34	33	0
507-C, fasted (no K)	60	101.0	77%	252	89%	33	0	48	19
507-D, fasted (higher Na, No Mg)	60	120.8	92%	257	90%	50	34	16	0
507-G (3.75 g O + 0.75 g X, fasted	60	95.6	72%	246	87%	23	19	40	18
X, fasted	60	131.9	100%	284	100%	100	0	0	0

Example 2.4: Testing Effect of Dilution Volume

Formulation 507-D having 50% sodium and tested at 60 mL volume was bioequivalent to Xyrem®, yet the four-cation blend of Example 2.2 having 49% sodium and tested at 240 mL volume was not bioequivalent. The difference between the two results is statistically significant and meaningful. To determine whether or how the volume of administration affects behavior of formulations, Formulation "O" was tested and compared to Xyrem® in three treatments fasted with 60 mL volume given, fasted with 240 mL volume, and fed with 60 mL volume. Thus, six treatments were administered in a crossover fashion involving 33 patients in a food effect bioequivalence study. The results are shown in FIG. **4** and Table 7.

There is little difference in the primary PK parameters (Cmax and AUC) as a result of volume of administration; however, there appears to be a difference in the mean plasma profile for Xyrem® at the two volumes when given fasted (FIG. **4**).

TABLE 7

Results of Study JZP258-101, n = 33 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	Mg•h/L)	ratio	Na	K	Ca	Mg
O, fasted	60	93.0	77%	238	95%	8	23	48	21
O, fasted	240	92.7	74%	233	90%	8	23	48	21
O, fed	60	63.0	52%	202	80%	8	23	48	21
X, fasted	60	120.5	100%	251	100%	100	0	0	0

TABLE 7-continued

Results of Study JZP258-101, n = 33 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	Mg•h/L)	ratio	Na	K	Ca	Mg
X, fasted	240	125.9	100%	258	100%	100	0	0	0
X, fed	60	68.6	57%	206	82%	100	0	0	0

Although the effect of dilution volume on food effect was not directly challenged in a single study, comparison of data from two crossover studies is possible for formulations "O" and Xyrem®. Table 8 shows the comparison of data from study JZP258-101 for 60 mL dilution volume and from study 13-010 Part 1 for 240 mL dilution volume. The results indicate that formulation "O" has a reduced food effect compared to Xyrem® and that, in both cases, the higher dilution volume has a smaller food effect.

TABLE 8

Comparison of Food Effect at 60 mL and 240 mL dilution

Treatment	Cmax (mg/L)		AUC (mg · h/L)	
Volume	60 mL	240 mL	60 mL	240 mL
O, fasted	93.0	102.3	238	239
O, fed	63.0	77.7	202	216
Ratio of O, fed to O, fasted	68%	76%	85%	90%
X, fasted	120.5	134.6	251	268
X, fed	68.6	84.9	206	233
Ratio of X, fed to X, fasted	57%	63%	82%	87%

In similar fashion, comparison of fasted data across studies can be done. FIG. **5**A shows the Cmax ratio as a function of the percent of calcium in the formulation. FIG. **5**B shows the Cmax ratio as a function of the percent of sodium in the formulation. The calcium model was arrived at by stepwise regression of main effect and interaction of calcium % and volume of administration using JMP software (SAS Institute). Volume of administration and its interaction were both dropped as insignificant terms. (An alternative model process employing calcium % and diluted concentration—which is volume-dependent—provided no better fit.) The result has significant lack of fit.

On the other hand, when sodium level and sodium diluted concentration (and interaction) are considered, a significantly better fit to results was obtained. All three terms were significant at 90% confidence or better, yet the main effect of diluted sodium concentration was least significant of the

three). Sodium level and its interaction with diluted sodium concentration were highly significant, respectively). That model fit is shown in FIG. **5**B.

Example 3: Expected Pharmacokinetics of Two Formulations Dosed 4 Hours Apart

The following proposed test treatment consists of administering formulation "O" of preceding examples and administering a second dose of formulation "507-D" 4 hours later. The reference treatment consists of Xyrem® given in the same fashion. Test and reference treatments have the same oxybate dose and are administered in 60 mL of water in the evening approximately two hours after dinner. Plasma is sampled at the same intervals as in preceding examples.

The outcome can be estimated by assuming additive contributions from each dose based on the single dose PK evaluations presented in preceding examples. The expected results are shown in FIG. **6** compared to those of the reference Xyrem® given under the same conditions.

Example 4: Microbial Challenge

This Example demonstrates that a mixed oxybate salt having low sodium displays acceptable resistance to microbial growth. A solution having, on a molar equivalents basis, 8% sodium, 23% potassium, 48% calcium, and 21% magnesium oxybate salts (Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$) with a pH value of 8 and a total concentration of 409 mg/mL oxybate salts was tested for antimicrobial effectiveness according to USP<51>. Individual samples were inoculated with each of five microorganisms and stored for 28 days at 20-25° C. At 7, 14, and 28 days microbial enumeration tests revealed effective reductions for all strains, as shown in Table 9 below.

TABLE 9

Microbial Effective
Test of 8% Na•GHB, 23% K•GHB,
48% Ca•$(GHB)_2$, and 21%
Mg•$(GHB)_2$ at 409 mg/mL
Log reduction in colony forming units/mL

Organism	Day 7	Day 14	Day 28
S. aureaus	>5.2	>5.2	>5.2
E. coli	>5.7	>5.7	>5.7
P. aeruginosa	>5.8	>5.8	>5.8
C. albicans	3.0	>5.6	>5.6
A. niger	2.6	3.6	>4.2

What is claimed is:

1. A method of reducing food effect due to administration of gamma-hydroxybutyrate (GHB) in a patient having cataplexy in narcolepsy or excessive daytime sleepiness in narcolepsy, comprising:

orally administering to a patient in need thereof a pharmaceutically effective amount of a pharmaceutical composition of GHB in a unit dosage comprising at least one salt of GHB and a pharmaceutically acceptable carrier within four hours after eating;

wherein the pharmaceutical composition of GHB has reduced food effect as measured by C_{max} compared to an equal dose of immediate release liquid solution of Na.GHB, wherein the pharmaceutical composition comprises: about 5% to about 10% of Na.GHB; about 20% to about 25% of K.GHB; about 45% to about 50% of $Ca.(GHB)_2$; and about 20% to about 25% of $Mg.(GHB)_2$.

2. The method of claim **1**, wherein the composition is administered with food, immediately after eating, up to 30 minutes after eating, or up to two hours after eating.

3. The method of claim **1**, wherein the composition provides an AUC when administered within four hours after eating that is 80%-95% of the AUC when the composition is administered while fasting.

4. The method of claim **1**, wherein the composition provides an AUC when administered within four hours after eating that is 85%-90% of the AUC when the composition is administered while fasting.

5. The method of claim **1**, wherein the composition provides a C_{max} when administered within four hours after eating that is 55%-80% of the C_{max} when the composition is administered while fasting.

6. The method of claim **1**, wherein the composition provides a C_{max} when administered within four hours after eating that is 60%-75% of the C_{max} when the composition is administered while fasting.

7. The method of claim **1**, wherein the composition provides a C_{max} that is less than the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses at least four hours after eating.

8. The method of claim **1**, wherein the composition provides a C_{max} that is less than the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses within four hours after eating.

9. The method of claim **1**, wherein the composition provides a C_{max} that is less than 60% the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses at least four hours after eating.

10. The method of claim **1**, wherein the composition provides a change in C_{max} when administered at least four hours after eating and within four hours after eating that is 10-60% less than the change in C_{max} of an equal dose of immediate release liquid solution of Na.GHB when administered in equally divided doses at least four hours after eating and within four hours after eating.

11. The method of claim **1**, wherein the pharmaceutical composition comprises 8% of Na.GHB; 23% of K.GHB; 48% of $Ca.(GHB)_2$; and 21% of $Mg.(GHB)_2$.

* * * * *

EXHIBIT 2

Guidance for Industry

Food-Effect Bioavailability and Fed Bioequivalence Studies

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

December 2002
BP

Guidance for Industry

Food-Effect Bioavailability and Fed Bioequivalence Studies

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Guidance For Industry[1]

Food-Effect Bioavailability and Fed Bioequivalence Studies

This guidance represents the Food and Drug Administration=s current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION

This guidance provides recommendations to sponsors and/or applicants planning to conduct food-effect bioavailability (BA) and fed bioequivalence (BE) studies for orally administered drug products as part of investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and supplements to these applications. This guidance applies to both immediate-release and modified-release drug products. The guidance addresses how to meet the BA and BE requirements in 21 CFR 320, 314.50 (d) (3), and 314.94 (a) (7) as they apply to oral dosage forms. This guidance provides recommendations for food-effect BA and fed BE study designs, data analysis, and product labeling. It also provides information on when food-effect BA and fed BE studies should be performed. [2]

II. BACKGROUND

Food effect BA studies are usually conducted for new drugs and drug products during the IND period to assess the effects of food on the rate and extent of absorption of a drug when the drug product is administered shortly after a meal (fed conditions), as compared to administration under fasting conditions. Fed BE studies, on the other hand, are conducted for ANDAs to demonstrate their bioequivalence to the reference listed drug (RLD) under fed conditions.

A. Potential Mechanisms of Food Effects on BA

[1] This guidance has been prepared by the Food Effect Working Group of the Biopharmaceutics Coordinating Committee in the Office of Pharmaceutical Science, Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA).

[2] See also the guidance for industry on *Bioavailablity and Bioequivalence Studies for Orally Administered Drug Products C General Considerations*.

Food can change the BA of a drug and can influence the BE between test and reference products. Food effects on BA can have clinically significant consequences. Food can alter BA by various means, including

- Delay gastric emptying
- Stimulate bile flow
- Change gastrointestinal (GI) pH
- Increase splanchnic blood flow
- Change luminal metabolism of a drug substance
- Physically or chemically interact with a dosage form or a drug substance

Food effects on BA are generally greatest when the drug product is administered shortly after a meal is ingested. The nutrient and caloric contents of the meal, the meal volume, and the meal temperature can cause physiological changes in the GI tract in a way that affects drug product transit time, luminal dissolution, drug permeability, and systemic availability. In general, meals that are high in total calories and fat content are more likely to affect the GI physiology and thereby result in a larger effect on the BA of a drug substance or drug product. We recommend use of high-calorie and high-fat meals during food-effect BA and fed BE studies.

B. Food Effects on Drug Products

Administration of a drug product with food may change the BA by affecting either the drug substance or the drug product. In practice, it is difficult to determine the exact mechanism by which food changes the BA of a drug product without performing specific mechanistic studies. Important food effects on BA are least likely to occur with many rapidly dissolving, immediate-release drug products containing highly soluble and highly permeable drug substances (BCS Class I) because absorption of the drug substances in Class I is usually pH- and site-independent and thus insensitive to differences in dissolution.[3] However, for some drugs in this class, food can influence BA when there is a high first-pass effect, extensive adsorption, complexation, or instability of the drug substance in the GI tract. In some cases, excipients or interactions between excipients and the food-induced changes in gut physiology can contribute to these food effects and influence the demonstration of BE. For rapidly dissolving formulations of BCS Class I drug substances, food can affect C_{max} and the time at which this occurs (T_{max}) by delaying gastric emptying and prolonging intestinal transit time. However, we expect the food effect on these measures to be similar for test and reference products in fed BE studies.

For other immediate-release drug products (BCS Class II, III, and IV) and for all modified-release drug products, food effects are most likely to result from a more complex combination of factors that influence the in vivo dissolution of the drug product and/or the absorption of the drug substance. In these cases, the relative direction and magnitude of food effects on formulation BA and the effects on the demonstration of BE are difficult, if not impossible, to predict without conducting a fed BE study.

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[3] See the guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*.

III. RECOMMENDATIONS FOR FOOD-EFFECT BA AND FED BE STUDIES

This section of the guidance provides recommendations on when food-effect BA studies should be conducted as part of INDs and NDAs and when fed BE studies should be conducted as part of ANDAs. For postapproval changes in an approved immediate- or modified-release drug product that requires in vivo redocumentation of BE under fasting conditions, fed BE studies are generally unnecessary.

A. Immediate-Release Drug Products

1. INDs/NDAs

We recommend that a food-effect BA study be conducted for all new chemical entities (NCEs) during the IND period.

Food-effect BA studies should be conducted early in the drug development process to guide and select formulations for further development. Food-effect BA information should be available to design clinical safety and efficacy studies and to provide information for the CLINICAL PHARMACOLOGY and/or DOSAGE AND ADMINISTRATION sections of product labels. If a sponsor makes changes in components, composition, and/or method of manufacture in the clinical trial formulation prior to approval, BE should be demonstrated between the to-be-marketed formulation and the clinical trial formulation.

Sponsors may wish to use relevant principles described in the guidance for industry on *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (SUPAC-IR guidance) to determine if in vivo BE studies are recommended. These BE studies, if indicated, should generally be conducted under fasting conditions.

2. ANDAs

In addition to a BE study under fasting conditions, we recommend a BE study under fed conditions for all orally administered immediate-release drug products, with the following exceptions:

- When both test product and RLD are rapidly dissolving, have similar dissolution profiles, and contain a drug substance with high solubility and high permeability (BCS Class I) (see footnote 3), or

- When the DOSAGE AND ADMINISTRATION section of the RLD label states that the product should be taken only on an empty stomach, or

- When the RLD label does not make any statements about the effect of food on absorption or administration.

B. Modified-Release Drug Products

We recommend that food-effect BA and fed BE studies be performed for all modified-release dosage forms.

1. INDs/NDAs

We recommend a study comparing the BA under fasting and fed conditions for all orally administered modified-release drug products.

When changes occur in components, composition, and/or method of manufacture between the to-be-marketed formulation and the primary clinical trial material, the sponsor may wish to use relevant principles described in the guidance for industry on *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls: In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation* (SUPAC-MR guidance) to determine if documentation of in vivo BE is recommended. These BE studies, if indicated, should generally be conducted under fasting conditions.

2. ANDAs

In addition to a BE study under fasting conditions, a BE study under fed conditions should be conducted for all orally administered modified-release drug products.

IV. STUDY CONSIDERATIONS

This section provides general considerations for designing food effect BA and fed BE studies. A sponsor may propose alternative study designs and data analyses. The scientific rationale and justification for these study designs and analyses should be provided in the study protocol. Sponsors may choose to conduct additional studies for a better understanding of the drug product and to provide optimal labeling statements for dosage and administration (e.g. different meals and different times of drug intake in relation to meals). In studying modified-release dosage forms, consideration should be given to the possibility that co-administration with food can result in *dose dumping*, in which the complete dose may be more rapidly released from the dosage form than intended, creating a potential safety risk for the study subjects.

A. General Design

We recommend a randomized, balanced, single-dose, two-treatment (fed vs. fasting), two-period, two-sequence crossover design for studying the effects of food on the BA of either an immediate-release or a modified-release drug product. The formulation to be tested should be administered on an empty stomach (fasting condition) in one period and following a test meal

(fed condition) in the other period. We recommend a similar, two-treatment, two-period, two-sequence crossover design for a fed BE study except that the treatments should consist of both test and reference formulations administered following a test meal (fed condition). An adequate washout period should separate the two treatments in food-effect BA and fed BE studies.

B. Subject Selection

Both food-effect BA and fed BE studies can be carried out in healthy volunteers drawn from the general population. Studies in the patient population are also appropriate if safety concerns preclude the enrollment of healthy subjects. A sufficient number of subjects should complete the study to achieve adequate power for a statistical assessment of food effects on BA to claim an absence of food effects, or to claim BE in a fed BE study (see DATA ANALYSIS AND LABELING section). A minimum of 12 subjects should complete the food-effect BA and fed BE studies.

C. Dosage Strength

In general, the highest strength of a drug product intended to be marketed should be tested in food-effect BA and fed BE studies. In some cases, clinical safety concerns can prevent the use of the highest strength and warrant the use of lower strengths of the dosage form. For ANDAs, the same lot and strength used in the fasting BE study should be tested in the fed BE study. For products with multiple strengths in ANDAs, if a fed BE study has been performed on the highest strength, BE determination of one or more lower strengths can be waived based on dissolution profile comparisons (for details see the guidance on *Bioavailablity and Bioequivalence Studies for Orally Administered Drug Products - General Considerations.*

D. Test Meal

We recommend that food-effect BA and fed BE studies be conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. A high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal is recommended as a test meal for food-effect BA and fed BE studies. This test meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.[4] The caloric breakdown of the test meal should be provided in the study report. If the caloric breakdown of the meal is significantly different from the one described above, the sponsor should provide a scientific rationale for this difference. In NDAs, it is recognized that a sponsor can choose to conduct food-effect BA studies using meals with different combinations of fats, carbohydrates, and proteins for exploratory or label purposes. However, one of the meals for the food-effect BA studies should be the high-fat, high-calorie test meal described above.

[4] An example test meal would be two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes and eight ounces of whole milk. Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

E. Administration

Fasted Treatments: Following an overnight fast of at least 10 hours, subjects should be administered the drug product with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

Fed Treatments: Following an overnight fast of at least 10 hours, subjects should start the recommended meal 30 minutes prior to administration of the drug product. Study subjects should eat this meal in 30 minutes or less; however, the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

F. Sample Collection

For both fasted and fed treatment periods, timed samples in biological fluid, usually plasma, should be collected from the subjects to permit characterization of the complete shape of the plasma concentration-time profile for the parent drug. It may be advisable to measure other moieties in the plasma, such as active metabolites, and sponsors should refer to the guidance on *Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations* for recommendations on these issues. Consideration should be given to the possibility that co-administration of a dosage form with food can alter the time course of plasma drug concentrations so that fasted and fed treatments can have different sample collection times.

V. DATA ANALYSIS AND LABELING

Food-effect BA studies may be exploratory and descriptive, or a sponsor may want to use a food-effect BA study to make a label claim.[5] The following exposure measures and pharmacokinetic parameters should be obtained from the resulting concentration-time curves for the test and reference products in food-effect BA and fed BE studies:

- Total exposure, or area under the concentration-time curve ($AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$)
- Peak exposure (C_{max})
- Time to peak exposure (T_{max})
- Lag-time (t_{lag}) for modified-release products, if present
- Terminal elimination half-life
- Other relevant pharmacokinetic parameters

Individual subject measurements, as well as summary statistics (e.g., group averages, standard deviations, coefficients of variation) should be reported. An equivalence approach is

[5] Regulations on labeling requirements for a drug product submitted in an NDA can be found in 21 CFR part 201.

recommended for food-effect BA (to make a claim of no food effects) and fed BE studies, analyzing data using an average criterion. Log-transformation of exposure measurements (AUC and C_{max}) prior to analysis is recommended. The 90 percent CI for the ratio of population geometric means between test and reference products should be provided for $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and C_{max} (see guidance for industry on *Statistical Approaches to Establishing Bioequivalence*). For IND or NDA food-effect BA studies, the fasted treatment serves as the reference. For ANDA fed BE studies, the RLD administered under fed condition serves as the reference treatment.

The effect of food on the absorption and BA of a drug product should be described in the CLINICAL PHARMACOLOGY section of the labeling. In addition, the DOSAGE AND ADMINISTRATION section of the labeling should provide instructions for drug administration in relation to food based on clinical relevance (i.e., whether or not the changes in systemic exposure caused by co-administration with food results in safety or efficacy concerns, or when there is no important change in systemic exposure but there is a possibility that the drug substance causes GI irritation when taken without food).

For an NDA, an absence of food effect on BA is not established if the 90 percent CI for the ratio of population geometric means between fed and fasted treatments, based on log-transformed data, is not contained in the equivalence limits of 80-125 percent for either $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) or C_{max}. When the 90 percent CI fails to meet the limits of 80-125 percent, the sponsor should provide specific recommendations on the clinical significance of the food effect based on what is known from the total clinical database about dose-response (exposure-response) and/or pharmacokinetic-pharmacodynamic relationships of the drug under study. The clinical relevance of any difference in T_{max} and t_{lag} should also be indicated by the sponsor. The results of the food-effect BA study should be reported factually in the CLINICAL PHARMACOLOGY section of the labeling and should form the basis for making label recommendations (e.g., *take only on an empty stomach*) in the DOSAGE AND ADMINISTRATION section of the labeling. The following are examples of language for the package insert:

> A food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that the C_{max} and AUC were increased 57% and 45%, respectively, under fed conditions. This increase in exposure can be clinically significant, and therefore [the drug] should be taken only on an empty stomach (1 hour before or 2 hours after a meal)
>
> A food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that the C_{max} was decreased 15% while the AUC remained unchanged. This decrease in exposure is not clinically significant, and therefore [the drug] could be taken without regards to meals.

An absence of food effect on BA is indicated when the 90 percent CI for the ratio of population geometric means between fed and fasted treatments, based on log-transformed data, is contained in the equivalence limits of 80-125 percent for $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}. In this case, a sponsor can make a specific claim in the CLINICAL PHARMACOLOGY or DOSAGE AND ADMINISTRATION section of the label that no food effect on BA is expected

provided that the T_{max} differences between the fasted and fed treatments are not clinically relevant. The following is an example of language for the package insert:

> The C_{max} and AUC data from a food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that exposure to the drug is not affected by food. Therefore, [the drug product] may be taken without regard to meals.

For an ANDA, BE of a test product to the RLD product under fed conditions is concluded when the 90 percent CI for the ratio of population geometric means between the test and RLD product, based on log-transformed data, is contained in the BE limits of 80-125 percent for AUC and C_{max}. Although no criterion applies to T_{max}, the T_{max} values for the test and reference products are expected to be comparable based on clinical relevance. The conclusion of BE under fed conditions indicates that with regard to food, the language in the package insert of the test product can be the same as the reference product.

VI. OTHER CONSIDERATIONS

A. Sprinkles

In NDAs, the labeling of certain drug products (e.g., controlled-release capsules containing beads) can recommend that the product be sprinkled on soft foods, such as applesauce, and swallowed without chewing. For the labeling to indicate that the drug product can be sprinkled on soft foods, additional in vivo relative BA studies should be performed by sprinkling the product on the soft foods to be listed in the labeling (test treatment) and comparing it to the product administered in the intact form (reference treatment), then administering both on an empty stomach.

In ANDAs, BE of the test to the RLD is demonstrated in a single dose crossover study. Both treatments should be sprinkled on one of the soft foods mentioned in the labeling, usually applesauce. The BE data should be analyzed using average BE and the 90 percent CI criteria should be used to declare BE. If there are questions about other foods, the design, or the analysis of such BE studies, the sponsors and/or applicants should contact the Office of Generic Drugs.

B. Special Vehicles

For NDAs, the labeling for certain oral solution products (e.g., cyclosporine oral solution, modified) recommends that the solution be mixed with a beverage prior to administration. The BA of these products can change when mixed with different beverages due to the formation of complex mixtures and other physical-chemical and/or physiological factors. NDA sponsors should contact the Office of Clinical Pharmacology and Biopharmaceutics to determine what data should be submitted to support labeling.

In ANDAs, BE of the test to the RLD is demonstrated in a single-dose crossover study. Both treatments should be mixed with one of the beverages mentioned in the labeling. Sponsors

should provide evidence that BE differences would not be expected from the use of other listed vehicles. The BE data should be analyzed using average BE, and the 90 percent CI criteria should be used to declare BE. If there are questions about other vehicles, or the design or analysis of such BE studies, the sponsors and/or applicants should contact the Office of Generic Drugs.

EXHIBIT 3

Notice of Allowability	Application No. 17/131,418	Applicant(s) Allphin et al.	
	Examiner CHRIS E SIMMONS	Art Unit 1629	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to the amendment filed 4/8/2022.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 51-61 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 10/12/2021.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/CHRIS E SIMMONS/ Examiner, Art Unit 1629	/JEFFREY S LUNDGREN/ Supervisory Patent Examiner, Art Unit 1629

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

The application has been amended as follows:

Claim 51, ***replace*** the term "administration of GHB" at line 2 with ---- administration of gamma-hydroxybutyrate (GHB) ----

Reasons for Allowance

The following is an examiner's statement of reasons for allowance: the prior art does not teach the presently-claimed methods provide the unexpected result that there is a reduced food effect in patients administered a composition having the claimed GHB salt mixture (as exemplified by Formulation O from the present specification) for the treatment of cataplexy or excessive daytime sleepiness with narcolepsy compared to patients administered an equal dose of immediate release liquid solution of Na*GHB (as exemplified by XYREM®). This result of the claimed methods was unexpected based on the prior art.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CHRIS E SIMMONS whose telephone number is (571)272-9065. The examiner can normally be reached M-F: 8-4:30p.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey S. Lundgren can be reached on (571)272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: https://patentcenter.uspto.gov. Visit https://www.uspto.gov/patents/apply/patent-center for more information about Patent Center and https://www.uspto.gov/patents/docx for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC)

at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service

Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/CHRIS E SIMMONS/
Examiner, Art Unit 1629

/JEFFREY S LUNDGREN/
Supervisory Patent Examiner, Art Unit 1629

EXHIBIT 4

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

JAZZ PHARMACEUTICALS IRELAND, LIMITED, **Plaintiffs,** **v.** **LUPIN INC. and LUPIN PHARMACEUTICALS, INC.,** **Defendants.**	**Civil Action No. 21-14271 (SRC)(JSA)** **Civil Action No. 22-2773 (SRC)(JSA)** **Civil Action No. 23-329 (SRC)(JSA)** **(CONSOLIDATED)** **(Filed Electronically)**

DECLARATION OF PANAYIOTIS P. CONSTANTINIDES, PH.D.

I, Panayiotis P. Constantinides, declare and state as follows:

I. Introduction

1. My name is Panayiotis P. Constantinides, and I have been retained by McGuireWoods LLP, counsel for Defendants Lupin Inc. and Lupin Pharmaceuticals, Inc. (collectively, "Lupin"), in the above-captioned matter to provide a technical overview of pharmacokinetics as it relates to oral drug administration.

2. I have personal knowledge of the facts contained in this Declaration, and, if called as a witness, I could and would testify competently thereto.

3. The technical overview contained in this Declaration is based on my years of education, research, and experience, as well as my investigation and study of the cited materials (referenced below).

II. Qualifications

4. I am an expert in the fields of drug delivery and development, and in particular, pharmaceutical formulations. A summary of my qualifications and credentials is summarized below and is set forth more fully in my current Curriculum Vitae (Exhibit 1).

5. I received a University Diploma in Chemistry from the National and Kapodistrian University of Greece in Athens, Greece in 1977. From there, I attended Brown University, where I received a Ph.D. in Biochemistry (physical) in 1983. After that, I pursued a Postdoctoral Fellowship in Pharmacology and Cancer Research at Yale University, which I completed in 1985, and continued as an Associate Research Scientist there for two years.

6. In 1987, I joined the biotech and pharmaceutical industries and progressed through a number of research and development positions of increasing responsibility with small biotech and large pharmaceutical companies, including SmithKline Pharmaceuticals (now GlaxoSmithKline) and Abbott Laboratories (working in the Pharmaceutical Products Division).

7. In 2004, I founded Biopharmaceutical & Drug Delivery Consulting, LLC, in Gurnee, Illinois, serving as Owner/President, providing consulting services to the biotech and pharmaceutical industries, and serving as an expert witness in pharmaceutical patent and breach of contract litigations.

8. In addition to my industrial appointments, I have held adjunct faculty positions with Roosevelt University, School of Pharmacy, Department of Biopharmaceutical Sciences, in Schaumburg, Illinois, the University of Washington, Department of Pharmaceutics, in Seattle, Washington, and the University of Tennessee, Department of Biochemistry, in Knoxville, Tennessee.

9. The areas of my research and experience when I was employed by various biotech and pharmaceutical companies, and as an independent consultant, include oral, parenteral, and topical drug delivery systems and formulations of New Molecular Entities (NMEs), as well as reformulations of same, and product line extensions of approved/marketed drugs, and biologics (particularly peptide and protein therapeutics).

10. My product development experience with formulation technologies and dosage forms of both water-soluble and poorly soluble drugs includes injectable ready to use solutions, suspensions, liposomes, emulsions, nanoparticles, as well as lyophilized powders for reconstitution, oral liquid, semi-solid and solid dosage forms (immediate and sustained/controlled release), self-emulsifying drug delivery systems, solid dispersions, and topical solutions, ointments, creams, foams, and gels.

11. With reference to oral formulation development, my involvement over the years has included assessing formulation physical/chemical stability and *in vitro* dissolution in simulated gastric fluid (SGF), as well as simulated fasted and fed state intestinal fluids. Included within my research and development responsibilities and as a consultant are oral absorption studies in animals (rodents, dogs, mini-pigs, and primates) and in humans to determine pharmacokinetic (PK) parameters and oral bioavailability, and food effects on these PK parameters.

12. I have also been involved in processing and manufacturing aspects of drug formulations, including manufacturing clinical trial material (CTM) batches, *in vitro* physicochemical characterization, and correlation to *in vivo* performance. I have further been involved in formulation strategies to address physical and chemical stability challenges with the API (Active Pharmaceutical Ingredient) during processing and upon storage, as well as *in vivo*

stability, such as pH-dependent and enzymatic degradation, particularly in the harsh environment of the gastrointestinal (GI) tract.

13. My expertise also extends to functional excipient development and qualification, including novel excipients for pharmaceutical development, and new as well as non-traditional uses of existing pharmaceutical excipients.

14. As set forth in more detail in my accompanying Curriculum Vitae, I have thirty-six (36) years of experience related to the development of pharmaceutical products in multiple disease indications with an in-depth understanding of drug formulation and delivery.

15. Beyond formulation development, my responsibilities over the years as Project Leader, Head of Research and Development, and consultant, have included analytical method development and validation, manufacturing and technology transfer, and in-process and finished product tests according to set specifications, including stability testing under ICH guidelines.

16. I have also been responsible for assessing, selecting, and applying enabling drug delivery technologies based on the physicochemical and biopharmaceutical properties of the API. Development and implementation of Targeted Product Profile (TPP) of New Molecular Entities and Product Line Extensions has been a key area of my involvement and responsibility.

17. With reference to the API, I have experience with various inorganic and organic salts, drug conjugates as well as non-covalent complexes and ion-pairs with lipid and polymeric excipients. My drug development experience, beyond branded drug products, includes generic drugs, particularly complex generics, as well as branded generics, also known as super generics.

18. My work both as a full-time employee with various biotech/pharma companies and as a consultant has contributed to several drug products currently on the market and in advanced clinical development.

19. I have authored/co-authored over 50 original research papers, review articles, book chapters, commentaries, and interview publications, and have delivered more than 100 invited talks worldwide in the areas of drug delivery and formulation development of small molecules and peptide/protein therapeutics, traditional and non-traditional uses of pharmaceutical excipients, pharmaceutical nanotechnology, and research and development strategy and collaboration models. I have also been an editorial board member and referee for multiple peer reviewed journals.

20. I am listed as an inventor/co-inventor on 17 U.S. patents, 4 European patents, 17 WO (World Intellectual Property) patents, and several additional patent applications.

21. I have received numerous awards and honors, including the Browne-Coxe Postdoctoral Fellowship at Yale University School of Medicine.

22. Additionally, I was elected an AAPS Fellow in 2010 and I have held various leadership roles within AAPS including Chair of the Formulation Design and Development section and Chair of the Lipid-Based Drug Delivery Systems and Nanotechnology Focus Groups.

23. In recognition of my work with pharmaceutical excipients, I received the 2021 IPEC Foundation Henk de Jong Industrial Award for Outstanding Achievements in Excipient Research and Innovation.

III. Technical Overview

24. Pharmacokinetics (PK) is the investigation of drug absorption, distribution, metabolism, and excretion, commonly referred to as "ADME." (Exhibit 2).

25. In the case of oral absorption, a drug is absorbed through the intestinal mucosa to reach its site of action. The mechanisms by which oral drugs are absorbed are by simple (passive) diffusion, active transport, facilitated diffusion, and endocytosis.

26. "Bioavailability" (BA) is defined as the fraction of unchanged drug that enters systemic circulation after administration that becomes available to produce the desired effect.

27. Intravenous (I.V.) administration of a drug provides 100% bioavailability. Oral administration usually provides less bioavailability than I.V. administration. This can be expressed as % BA = AUC oral / AUC I.V. x 100, where "AUC" is the area under the plasma concentration vs. time curve. (Exhibit 2).

28. Both drug/formulation and physiological factors can affect pharmacokinetic parameters, including the AUC and "Cmax," which is the maximum (peak) concentration in the plasma concentration vs. time curve.

29. Drug/formulation factors include the molecular weight of the drug, degree of drug dissolution from the formulation/dosage (which follows the order: solution > suspension > capsule > tablet), chemical instability in gastric pH, and first pass metabolism, which reduces oral bioavailability. (Exhibit 3).

30. Physiological factors include blood flow to absorption site (greater blood flow increases bioavailability), surface area available for absorption, rate of gastric emptying (rapid gastric emptying leads to fast transit to intestine), and gastrointestinal (GI) pH. (Exhibit 2, 3).

31. "Bioequivalence" (BE) generally is defined as two drugs/drug products showing comparable bioavailability at similar times (Tmax) to achieve peak blood concentration (Cmax). U.S. Patent No. 11,426,373 ("the '373 patent") defines bioequivalence as:

> a formulation and/or pharmaceutical composition that is therapeutically equivalent to a reference product (e.g. Xyrem®) when given under the same conditions in a pharmacokinetic evaluation conforming to FDA Guidance on Bioequivalence Testing; regardless of biopharmaceutical class. A value that is "bioequivalent", as used herein, is meant to refer to a pharmacokinetic value (such as the Cmax or AUC of a formulation described herein) that exhibits substantially similar pharmacokinetic profiles or therapeutic effects. Bioequivalence may be demonstrated through several in vivo and in vitro methods. These methods may

> include, for example, pharmacokinetic, pharmacodynamic, clinical and in vitro studies. In some embodiments, bioequivalence may be demonstrated using any suitable pharmacokinetic measures or combination of pharmacokinetic measures known in the art, including loading dose, steady-state dose, initial or steady-state concentration of drug, biological half-life, elimination rate, area under the curve (AUC), clearance, the peak blood or plasma concentration (Cmax), time to peak concentration (Tmax), bioavailability and potency. In some embodiments, a value is bioequivalent to a reference pharmacokinetic value when the geometric mean of the AUC and/or the Cmax is between 80% and 125% (e.g., at 90% confidence interval) of the reference pharmacokinetic value. (Exhibit 4).

32. For a clinical *in vivo* bioequivalence assessment, it is imperative that the dosing regimen (i.e., the amount, time, and number of times a specific quantity of the drug is administered), is clearly defined and is the same for the two drugs/drug products. Other study design recommendations can be found in the FDA Guidance for Industry. (Exhibit 5).

33. "Food effect" on drug absorption is very important in drug development. Food effect can be described as the effect of food on the rate and extent of absorption of a drug when the drug product is administered shortly after a meal (fed conditions), as compared to administration under fasted conditions." (Exhibit 3). It can be mediated by various mechanisms that include solubility enhancement, change in the GI pH and mobility, delayed stomach emptying, increased bile salt concentration, and direct interactions with the drug. (Exhibit 3).

34. There are three scenarios involving the effect of food on drug absorption: (a) no food effect, where the same AUC and Cmax values are obtained for the fasted and fed states; (b) positive food effect, where the AUC and Cmax values for the fed state are greater than the corresponding values for the fasted state; and (c) negative food effect, where the Cmax and AUC values for the fed state are lower than the corresponding values for the fasted state.

35. It should also be emphasized that variability is generally observed between fasted and fed states, and can be contributed to various factors, including: (a) anatomical and physiological factors (GI transit time, mobility, pH, fluid volume and composition, plasma

proteins, transporters); (b) demographical and genetic factors (age, height, weight, gender, genetics, disease); (c) drug specific factors (molecular weight, charge, solubility and partition coefficient, dosing frequency, volume of distribution, bioavailability, particle size, surface area, protein binding), and (d) formulation factors (immediate vs. modified release, liquid vs. solid dosage forms, excipients present that can enhance drug solubility and/or intestinal mucosa permeability). (Exhibit 3).

36. Prediction of whether an orally administered drug product will show a food effect in humans can be challenging. To this end, recently developed pharmacokinetic modeling tools can be used to predict food effect in humans. One such modeling tool is the Physiologically Based Pharmacokinetic (PBPK) model, which integrates *in silico, in vitro*, and preclinical *in vivo* data. (Exhibit 3).

37. The Examples from the '373 patent describe several different clinical bioequivalence evaluations. Example 2.1 compares a mixed-salt GHB formulation of Formulation "O" with Xyrem® (called Formulation "X"). According to the patent, "[t]he study was compliant with the FDA guidance for food effect studies ('Guidance for Industry: Food-Effect bioavailability and Fed Bioequivalence Studies', FDA December 2002), incorporated herein by reference in its entirety." (Exhibit 4).

38. Under this guidance, as described above, the dosing regimen (i.e., the amount, time, and number of times a specific quantity of the drug is administered), must be clearly defined and should be the same for the two drugs/drug products.

39. With respect to the timing of doses for food effect studies:

> **Fasted Treatments**: Following an overnight fast of at least 10 hours, subjects should be administered the drug product with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as

> desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.
>
> **Fed Treatments**: Following an overnight fast of at least 10 hours, subjects should start the recommended meal 30 minutes prior to administration of the drug product. Study subjects should eat this meal in 30 minutes or less; however, the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study. (Exhibit 5).

40. Thus, there are directions for the timing of doses in a food effect study. For studies attempting to compare the effect of food on one pharmaceutical composition relative to another, the study must be performed on each under the same conditions and timelines.

41. For example, if the fed dosing regimen for Drug Product A is one 10-mg drug dose thirty minutes after eating, then Drug Product B must also be dosed as one 10-mg drug dose thirty minutes after eating.

42. If Drug Product A was dosed thirty minutes after eating and Drug Product B was dosed five hours after eating, a proper comparison of the relative effect of food on the two drug products could not be made. The same is true of splitting a dosage amount into separate aliquots.

43. For example, if Drug Product A is dosed as one 10-mg drug dose thirty minutes after eating, and Drug Product B is dosed as two 5-mg drug doses (one 30 minutes after eating and the next 2 hours after eating), a proper comparison of the relative effect of food on the two drug products could not made.

44. In sum, a comparison of the food effect on two different drug products requires that they be tested under the same conditions with respect to dosage and timing of administration.

I declare under penalty of perjury the foregoing is true and correct.

Executed this 4th day of October, 2023.

Panayiotis P. Constantinides, Ph.D

EXHIBITS

1. Curriculum Vitae of Panayiotis P. Constantinides, Ph.D
2. Leslie Z. Benet and Parnian Zia-Amiphosseini. Basic Principles of Pharmacokinetics. Toxicologic Pathology (1995), Vol. 23: 115-123.
3. Tycho Heimbach, Binfeng Xia, Tsu-han Lin and Handan He. Case Studies for Practical Food Effect Assessments across BCS/BDDCS Class Compounds using *In Silico*, *In Vitro* and Preclinical *In Vivo* Data. AAPSJ (2013), Vol. 15:143-158.
4. U.S. Patent No. 11,426,373.
5. FDA Guidance for Industry, Food-Effect Bioavailability and Fed Bioequivalence Studies, December 2002.

EXHIBIT 1

CURRICULUM VITAE OF PANAYIOTIS P. CONSTANTINIDES

95 Berkshire Court, Gurnee, IL 60031- 6226, USA
Office : + 1 (847) 599-9496 ; Mobile: +1 (224) 430-0383; E-mail: constantinpp@aol.com; ppconstantinides@bpddc.com

EXECUTIVE SUMMARY

Career Objectives: *Expand therapeutic utility and market value of small molecule and macromolecule (biologics) drugs, approved and New Molecular Entities (NMEs), from innovative drug formulation and delivery technology, product development and commercialization, life-cycle management, strategic partnerships and business development perspectives.*

Education: University Diploma/B.Sc. *Chemistry*, 1977 (Athens University), Ph.D., *Biochemistry*, 1983 (Brown University), Postdoctoral Training, *Pharmacology/Cancer Research,* 1983-1985 (Yale University).

Industrial Experience: Big Pharma (8 years), Generic/Specialty Pharma/Drug Delivery/Biotech (9 years); consultant (18 years).. Experience in both pharmaceutical and nutraceutical development. ***Dosage Forms****:* parenteral (intravenous solutions, liposomes, micelles, emulsions, micro-/nanoparticles, cyclodextrins; liquid and lyophilized formulations); oral hard and soft gelatin capsules (liquid, suspension, semi-solid fill); immediate and controlled release tablets and capsules; nasal sprays and topical formulations (foams, gels, creams, ointments). ***Drug Molecules****:* Small molecule and macromolecules (peptides, proteins and vaccines); BCS II/IV and BCS III molecules; approved/marketed drugs and New Molecular Entities (NMEs). ***Therapeutic Areas****:* Cancer, Immune System, Cardiovascular, CNS, GI, Infectious Diseases, Endocrinology/Metabolic, Inflammation and Tissue Repair. *Regulatory Filings:* IND, ANDA, 505 (b) (2), NDA.

Academic Experience: Teaching and Research Assistant, Postdoctoral Fellow, Associate Research Scientist, Adjunct Assistant Professor of Biochemistry, Adjunct Associate Professor of Pharmaceutics, Professor in-Charge/Instructor of special short courses, Affiliate Associate Professor and Professor of Biopharmaceutical Sciences. Delivered lectures in physical chemistry, biochemistry, pharmaceutics and drug delivery, biotechnology, nanotechnology, entrepreneurship, and R&D management. Advisor to academic inventors and founders of university spin-off companies.

Management Experience: Scientific leader and seasoned executive and experienced consultant with excellent organization, interpersonal and communication skills. Results oriented, resourceful and able to interface with the right people/groups both internally and externally to achieve timely results. Timely and focused planning and execution with problem-solving skills and assertiveness in decision making. ***Responsibilities***: Founder Owner/President, Biopharmaceutical & Drug Delivery Consulting, LLC, Vice President of R&D, Director of Research, Section Head, Team Leader/Project Manager in the areas of Discovery Research, Technology and Intellectual Property Development and Management, and Product Development (formulation and method development and validation, scale-up and manufacturing and technology transfer). Developed and managed research and development collaborations and with universities, big pharma, biotech/drug delivery companies, contract research and contract development and manufacturing organizations.

Publications/Patents : 30 original research papers, 1 Commentary, 13 review articles/book chapters, 2 theme issues editor, 3 interview publications, 1 professional development article, 20 poster presentations, 103 invited talks, 17 US Patents (6,008,192; 6,458,373; 6,479,540; 6,660,286; 6,667,048; 8,241,664;8,481,084; 8,492,369;8,536,650; 8,778,916; 8,778,917, 8,828,428, 10,245,273; 10,307,441, 10,463,689, 11,179,402, 11,179, 403), 4 European Patents (1871384,1460992, 2056835,2167069), 17 WO patents (93/02664, 93/02665, 94/08603, 94/08605, 94/08610, 94/19000, 94/19001, 94/19003, 95/08986, 98/40051, 03/047494, 03/047493, 03/057128, 03/057193, 06/113505, 07/117556A2,15/100406), 5 US Patent Applications (US20080317844, US2007/0224293/ 0231412,/0281025, US2011/0086069) and 2 other (AU 02/9482601 and US 03/087954). Editorial board member and referee for peer-reviewed journals and invited speaker in 103 presentations at national and international scientific and business meetings. Since 2004, serves as Expert Witness (consulting and testifying expert) in patent litigation and other pharmaceutical cases.

Professional Associations and Honors: American Chemical Society, Controlled Release Society and American Association of Pharmaceutical Scientists (AAPS). AAPS Fellow, Past Chair of the Formulation Design and Development (FDD) Section, the Lipid-Based Drug Delivery Systems Focus Group and the Nanotechnology Focus Group of AAPS. Member spotlight in the April 2015 issue of AAPS News Magazine. Organizer/Chair/Moderator/Speaker, national and international biomedical, pharmaceutical and nanotechnology conferences, special workshops and short courses. Advisory Board member of university spin-offs and contract development and manufacturing organizations. Editor, AAPS Open journal. Recipient of various awards and recognitions.

EDUCATION AND WORK EXPERIENCE

Education

1983 - 1985 **Postdoctoral Fellowship, Pharmacology and Cancer Research,** *Yale University*, Department of Pharmacology and Cancer Center , School of Medicine, New Haven, Connecticut.

1977 - 1983 **Ph.D, Biochemistry (Physical),** *Brown University***,** Chemistry Department Providence, Rhode Island. Thesis Title: *"Physical Properties of Long-Chain Fatty Acyl-CoAs."* Advisor: Professor Joseph M. Steim.

1973 - 1977 **University Diploma (B.S.), Chemistry**, *National and Kapodistrian University of Greece*, Athens, Greece.

Research and Development Interests/Areas of Involvement

Preformulation : API salt selection, cocrystal and polymorph screening, solubility studies in aqueous solutions as a function of pH and in biorelevant media, API excipient compatibility studies.

Parenteral drug development: small molecules and biologics (peptides/proteins, vaccines, nucleic acids) using liquid and lyophilized formulations, solutions, suspensions and nanosuspensions, liposomes, micelles, emulsions, and other lipid and polymeric nanoparticles.

Topical drug development: solutions, liposomes, emulsions, creams, gels, foams, ointments shampoos and lotions.

Oral formulation development of BCS II, III and IV, small molecules and macromolecules (peptides/proteins) using enabling drug delivery technologies to improve drug solubility and/or intestinal permeability. Oral Dosage Forms: *liquid, aqueous and non-aqueous* (solutions, suspensions, emulsions, self-emulsifying drug delivery systems, such as SEDDS/SMEDDS/SNEDDS), *semi-solid* using high m.p. lipid excipients filled into a hard or soft gelatin capsules; *solid:* tablets and capsules, immediate, sustained and controlled release using multiparticulate dosage forms such as granules and pellets.

Early formulation development with drug discovery compounds to improve drug solubility and/or permeability limitations for preclinical toxicology and Pharmacokinetic (PK) Studies and Proof-of-Concept (POC) studies in humans.

Life cycle management strategies and product line extensions with marketed drugs.

Particle Engineering in API and Drug Product Design; Combination drug products.

Pharmaceutical applications of nanotechnology.

Sustained and Controlled release technologies and dosage forms.

Generic drug development, particularly with branded generics and 505 (b) (2) filing.

Processing and manufacturing aspects of drug formulations (process development, validation and technology transfer), test methods and specifications and strategies to address physical and chemical stability issues for the drug substance and drug product.

Functional excipient development and qualification (DMF Type IV); novel excipients for pharmaceutical development and new uses of pharmaceutical excipients.

Lipid- and/or polymer-based micro- and nanoparticulate systems for targeted drug delivery and controlled release.

Development of lipidic and polymeric drug complexes and conjugates for oral and parenteral administration.

Quality-by-Design (QbD) applications in drug formulation and process development and optimization.

Experience

Industrial Research & Development

2004 - present **Founder and Principal/President, Biopharmaceutical & Drug Delivery Consulting, LLC Gurnee, Illinois (website: www.bpddc.com)**

Areas of Consulting: Drug product and drug delivery technology development. Chemistry, Manufacturing and Controls (CMC) aspects for small molecule and macromolecule drugs (peptides, proteins, vaccines and nucleic acids). Reformulations of marketed drugs and product line extensions. Scientific, strategic and business assessment (due diligence) of drug product candidates and delivery technologies along with in-/out-licensing recommendations. Identifying, structuring and executing milestone-driven research and development collaborations with corporate partners. Biomedical applications of nanomaterials and nanoparticles. Development and qualification of novel excipients and/or new uses of excipients for pharmaceutical development (DMF Type IV). Intellectual property development strategies and assistance with patent filings and expert witness in patent litigation cases. Development and teaching of short courses for industrial and academic parties.

Clients: biotech and pharmaceutical companies, drug discovery and development companies, university spin-offs and start-ups, generic/specialty pharma, animal health companies, nutraceutical, cosmeceutical, chemical and nanotechnology companies, excipient vendors, contract development and manufacturing organizations (CDMOs), academic institutions, management consulting companies, venture capital and other investment firms, patent law firms and expert witness service organizations.

Therapeutic Areas : Cancer, Cardiovascular, CNS, Endocrinology, Infectious Diseases, Inflammation and Tissue Repair, Metabolic Disorders, Cell Therapies and other.

Dosage forms: parenteral/intravenous, small and large volume parenteral solutions, suspensions, emulsions, lipid and polymeric micro- and nanoparticles; polymeric microspheres, amorphous solid dispersions, oral solid (immediate and controlled release), semi-solid and liquid formulations; topical solutions, liposomes, emulsions, gels, creams, foams and ointments.

Current and Past Projects (working with internal R&D and/or external CROs/CMOs)

Assists several early stage drug discovery companies with diverse product portfolios and targeted disease areas on the execution of their business plan and provides technical input and direction on product development particularly as related to drug delivery, formulation and analytical development, in vitro/ in vivo performance evaluations, Chemistry, Manufacturing and Controls (CMC) for regulatory compliance and filings.

Working with a virtual company since 2004, intimately assisted with the development of a proprietary oral lipid-based technology for lymphatic delivery and absorption from the discovery phase to its progression to advanced clinical development and NDA submission of a drug that is marketed in USA as injectable or topical drug product. This new oral drug product (softgel) was approved by the FDA in 2019 and granted 3-year market exclusivity. Co-inventor of the company's intellectual property portfolio and contributor to patent filing and prosecution.

Provides technical guidance to a pharmaceutical company on the development of a combination parenteral drug product.

Assists specialty pharma companies on the CMC aspects of generic and novel formulations of marketed drugs for ANDA and 505(b) (2) filings.

Provided strategic and technical input to a manufacturer of capsule dosage forms using lipid-based systems.

Provided strategic, technical and business development consulting to a vendor of pharmaceutical excipients.

Serves as a consultant and advisor on chemistry, formulation, manufacturing and controls (CMC) aspects of new molecule entities developed by academic institutions and funded by NIH SBIR/STTR grants.

Working with patent attorneys, assists client companies as a technical expert and/or inventor with the drafting of patent applications, responses to patent office actions and other patent prosecution aspects.

Working with patent law firms and expert witness service organizations, serves as expert witness in patent litigation, breach of contract and other cases, on behalf of the plaintiff(s) or defendant(s) dealing with pharmaceutical formulations, and drug delivery technologies and manufacturing of dosage forms, for injectable, oral and topical drug products. oral : liquid, aqueous and non-aqueous (solutions, suspensions, emulsions, self-emulsifying drug delivery systems SEDDS/SMEDDS/SNEDDS) and where the aqueous oral liquids are filled into vials or bottles and the non-aqueous liquids into a hard or soft gelatin capsule; *semi-solid* using high m.p. lipid excipients and filled into a hard or soft gelatin capsule; *solid:* tablets and capsules, immediate, sustained and controlled release, multiparticulate dosage forms such as granules and pellets; injectables: solutions, lipid nanoparticles, micro- and nanodispersions (liposomes, emulsions and nanoemulsions, suspensions and nanosuspensions, polymeric microspheres), lyophilized powders for reconstitution; topical: solutions, creams, gels, foams and ointments.
Number of expert witness cases as of February 2023: 18 (3 are ongoing). Testified in 7 depositions, 1 arbitration hearing and 1 trial.

Provides on-site training seminars to interested parties on delivery and formulation development aspects of challenging molecules (small molecules and peptides) for oral, parenteral and topical dosage forms.

Provided strategic and scientific direction to an established chemical company on several healthcare applications of a GRAS nanomaterial, particularly in the areas of infectious diseases and metabolic disorders.

Working with internal and external groups and senior management, timely and effectively addressed formulation development, physical/chemical stability and scale up and manufacturing issues with proprietary new chemical entities, further advancing their clinical development.

Prepared and submitted to senior management of a client company, a technical assessment of an outside developed formulation/drug delivery technology as an in-licensing opportunity.

Assisted a contract research organization that provides analytical and formulation support services with strategic and technical guidance.

Assisted a large diversified company on strategic plans and provided technical direction on product development and commercialization aspects of a new drug delivery technology.

Assisted an investment firm in their due diligence process and acquisition of a privately held biopharma company.

Developed and served as professor in-charge of a new short course for an academic institution on the Formulation and Drug Delivery Applications of Nanoparticles.

Developed and served as instructor of a Biotechnology Laboratory Operational Management short course offered by an academic institution.

2017-present **Smart Health Activator – member of the Ops Team** (Operations Team). A non-profit organization advancing commercialization of biotechnology being developed by Midwest Universities. Assists on due diligence matters for new molecular entities, formulation, delivery and development aspects.

2003 –2004 **Vice President, Research and Development, Morton Grove Pharmaceuticals, Vernon Hills, Illinois.** Reported directly to the President & CEO.

Led all internal and external product development activities in the areas of generic oral liquids, suspensions, syrups, inhalation solutions, nasal sprays and topical formulations (shampoos, lotions). Direct reports included: formulation and analytical method development and validation, process and instrument/computer validation.

Major Accomplishment: Instrumental in revamping the company's R&D efforts and building the team.

2001- 2003 **Vice President, Research & Development (1/01-7/02) and Consultant (8/02-12/02) DOR BioPharma, Inc (formerly ENDOREX Corporation), Lake Forest, Illinois.** Reported directly to the President & CEO.

Major Accomplishments:

Expanded the company's R&D team including outside consultants.

Streamlined resources and focused R&D activities of the company.

Managed R&D collaborations with two major pharmaceutical companies.

Managed contract manufacturing, stability testing and regulatory filings of OrBec™ (oral beclomethasone dipropionate, IR and CR tablet) a Phase II/III drug product for Graft-vs-Host Disease (GVHD) and Grohn's disease.

Led company's vaccine program (tetanus and influenza) using lipid nanoparticle and microparticle formulation approaches along animal immunization studies upon subcutaneous, peroral and nasal administration.

Expanded company's drug delivery platform and intellectual property portfolio. Created 3 new technology platforms: LPM™ (lipid polymer micelles) for enhancing the intestinal absorption of water-soluble drugs/peptides and LPE™/PLP™ (lipid polymer emulsions/polymer lipid particles) for enhancing the solubilization and oral absorption of water-insoluble drugs. Preclinical proof-of-concept of enhanced oral bioavailability has been demonstrated with leuprolide and paclitaxel, respectively.

Primary inventor of four WO patents and presented company's technologies and product portfolio at six national/international meetings and to business and financial communities.

In July of 2002 after the company adapted the implementation of a major restructuring and downsizing plan, served as a consultant of Oradel Systems Inc. a subsidiary of DOR BioPharma to further develop and/or out license these technologies to big Pharma or other drug delivery companies.

1997-2000 **Director of Research, SONUS Pharmaceuticals, Bothell, Washington.** Reported directly to the President & CEO**.**

Major Accomplishments:

Established the company's drug delivery program and expanded its technology base and intellectual property portfolio. Created and developed the company's TOCOSOL™ drug delivery technology.

Leading a team of scientists, developed a novel, stable, filter-sterilizable and efficacious injectable nanoemulsion of paclitaxel (TOCOSOL™-Paclitaxel) from idea inception to scale up, preclinical evaluation and initiation of clinical studies.

Made significant contributions to the company's efforts to build research and development collaborations with big pharmaceutical companies.

1995-1997 **Section Head, Formulation Development, Pharmaceutical and Analytical Research and Development, Pharmaceutical Products Division, Abbott Laboratories, North Chicago, Illinois.**

Major Accomplishments:

Led project activities on formulation development, scale-up and manufacturing of oral liquid solutions, liquid-filled soft gelatin and semi-solid-filled hard gelatin capsule formulations of cyclosporine.

Contributed to the commercialization of a generic cyclosporine formulation from preclinical development to clinical manufacturing and bioequivalency testing along with all necessary material (CMC section) for the ANDA. Gengraf® approved by the FDA on May 15, '00 as cyclosporine capsules, USP (bioequivalent to Novartis Neoral®).

Led prior art search and patent filing strategy on generic cyclosporine formulations that resulted in 2 major patent filings. Major inventor on the Gengraf™ patent (US 6, 008, 192, December 28, 1999).

Key member of a multi-disciplinary Drug Delivery Technology Evaluation Team within the Formulation Center that interfaced with Corporate Licensing and Business Development to evaluate outside developed technologies with potential applications to Abbott's compounds.

1994-1995 **Senior Investigator/Team Leader, Pharmaceutical Product Development, Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania.**

Major Accomplishment:

Led a project team that developed a Phase I formulation of a water-soluble molecule which in preclinical studies in dogs and primates
showed enhanced oral absorption compared to a solution formulation.

1990 -1994 **Senior Investigator, Drug Delivery Department, Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania.**

Major Accomplishments:

Interfaced between Discovery Programs and Development Project Teams within SmithKline Beecham, to identify early development candidates and address pre-formulation, formulation development and drug delivery issues with various preclinical compounds, development compounds and product line extensions.

Established a drug delivery program and a useful lipid microemulsion database using model and proprietary water-soluble molecules/peptides from idea inception and formulation development to preclinical evaluation for toxicity and oral bioavailability assessment.

Creation of a strong intellectual property portfolio in the area of oral delivery of water-soluble molecules/peptides using microemulsions. Invented and developed (hands-on)

lipid microemulsion formulations that significantly improved the oral absorption of poorly absorbed drugs/peptides in animal models..

Led research activities on the feasibility of liposomal and emulsion formulations to improve the efficacy and reduce toxicity of antitumor and antiviral drugs upon parenteral administration and comparison to drug solutions or suspensions.

Provided several technical appraisals/reports on outside developed drug delivery systems/technologies with recommendations for potential licensing.

1988 - 1989 **Project Leader/Liposome Technology Development, Lipogen Inc. Knoxville, Tennessee.**

Major Accomplishments :

Provided technical and management support of a group of scientists on the development of a homogeneous liposome-based immunoassay which allows rapid qualitative (yes/no), or quantitative detection of a variety of classes of analyte i.e. therapeutic drugs in biological fluids (serum or urine).

Interfaced group's activities to those of the Marketing and Quality Control departments.

Developed a homogeneous liposome-based immunoassay for the detection and quantification of therapeutic drugs and other analytes in biological fluids.

1987-1988 **Research Scientist and Senior Research Scientist/Formulations, Lipogen Inc. Knoxville, Tennessee.**

Major Accomplishments:

Research and Development in the area of drug delivery systems using liposomes and other lipid-based carriers. Hands-on experience with drug-liposome formulation and physical characterization, lipid-antibody conjugation (immunoliposomes), design and formulation of phospholipid and other lipophilic prodrugs in liposomes, and stability, sterilization and scale-up of liposomal drugs.

Developed a lipid admixture for the solubilization of lipophilic and other hydrophobic compounds that can be administered parenterally or orally.

Liposome formulation and characterization, kinetic and thermodynamic studies of a homogeneous liposome-based immunoassay.

As a principal investigator, prepared and submitted to NIH two Small Business Innovation Research (SBIR) Phase I grants, on a) Formulation and Antitumor Activity of Lipophilic Methotrexate and, b) Target-Specific Delivery of Lipophilic Anticancer Drugs. Both were highly rated but not approved for funding.

1976 **Industrial Internship, Kyknos Canning Company, Nafplion, Greece.**

Work involved chemical analysis of canned fruits and vegetables such as acidity, solid content and other quality tests.

Academic Research & Teaching

2014 – 2016 **Affiliate Professor of Biopharmaceutical Sciences, Roosevelt University, College of Pharmacy, Schaumburg, Illinois.**

Member of the Biopharmaceutical Sciences Research Council.

2012-2013 **Affiliate Associate Professor of Biopharmaceutical Sciences, Roosevelt University, College of Pharmacy, Schaumburg, Illinois.**

Member of the Biopharmaceutical Sciences Research Council.

2007-2009 **Professor in-charge, University of Wisconsin, School of Pharmacy, Extension Services in Pharmacy.**

Coordinator and co-instructor of the annual short course on "Nanoparticles: Applications in Formulation and Drug Delivery".

1998 - 2000 **Associate Professor (Affiliate), Department of Pharmaceutics, University of Washington, Seattle, Washington.**

Co-instructor in a Pharmaceutical Biotechnology course and career mentor for graduate students. Research collaborations in drug transport and delivery.

1987- 1989 **Assistant Professor (Adjunct), Biochemistry Department, University of Tenessee, Knoxville, Tennessee.**

Co-instructor in a physical chemistry course (graduate level). Topics covered: lipid and membrane dynamics, biological spectroscopy (NMR, EPR, IR and Raman) and liposome technology.

1985 - 1987 **Associate Research Scientist, (Equivalent to Assistant Research Professor), Department of Pharmacology and the Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut.**

Research focused on the interaction of anthracyclines with lipid bilayers using Differential Scanning Calorimetry, as well as on size characterization of liposomes using Sedimentation Field Flow Fractionation, Electron Microscopy and Gel-Filtration.

1983 - 1985 **Postdoctoral Fellow, Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut.**

Reseach focused on: a) adriamycin-induced fusion of liposomes using EPR-spin-labelling, DSC, and Electron Microscopy; b) location of anthracyclines in lipid bilayers by paramagnetic quenching studies; and c) spin-trapping studies of hydroxyl and superoxide free radicals generated by the bioactivation of anticancer antibiotics.

1978 - 1980 **Research Assistant, Brown University, Providence, Rhode Island.**

Thesis reseach on the physical properties of long-chain fatty acyl-CoAs using Surface Tension, Conductivity, Fluorescence and Analytical Ultracentrifuge. Early work involved enzymatic studies with membrane-bound acyltransferace in a cell-free system to

understand how the activity of the enzyme is controlled by the physical state of the lipid bilayer.

1977 - 1983 **Teaching Assistant, Chemistry Department and Division of Biology and Medicine, Brown University, Providence, Rhode Island.**

Assisted in the teaching of Physical, Organic Chemistry and Biochemistry. Responsibilities included lectures on concepts and techniques pertaining to laboratory experiments, and preparation and supervision of laboratory sections and evaluation of student progress by grade assignment.

1976-1977 **Teaching Assistant, Inorganic Chemistry Department, Athens University, Athens, Greece.**

Duties included preparation and supervision of laboratory sections.

1977 **Part-time Teacher in Chemistry, Saint John Institute, Limassol, Cyprus.**

Preparation of high school students for university entrance examinations (G.C.E. level).

Special Skills

Technical

In depth-knowledge of the formulation and drug delivery science. Core competency: physical chemistry and biopharmaceutics of drug delivery systems with research and development expertise in lipid-based systems.

Product development experience (formulation, analytical and process development and manufacturing) of parenteral, oral and topical formulations.

Working knowledge of colloid and surface chemistry techniques, spectroscopic and bioanalytical methods, lipid and membrane biochemical and biophysical methodologies.

Computer skills : MacIntosh and IBM/PC using several programs, such as, Sigma Plot, Prism, Microsoft Office (Word, Excel, Project and Power Point).

Management

Supervisory and Project Management skills. The background of people supervised include: chemists, chemical engineers, biochemists, biologists and pharmacists at a B.Sc, M.Sc and Ph.D levels, as well as, undergraduate and graduate students in the aforementioned disciplines. Experienced in both line and matrix management. Proactive and coaching management style. Results oriented with excellent organization and communication skills.

Networking skills . Ability to interface with various levels of management and groups both internally and externally and achieve results working with multiple teams/disciplines.

Honors and Awards

9/73 - 6/77 Fellowship for Academic Excellence, National Fellowship Foundation of Greece.

9/84 - 9/85 Brown-Coxe Postdoctoral Fellowship, Yale University School of Medicine.

6/85 - present	Invited reviewer of research papers and review articles published in various Biochemical, Pharmaceutical and Medical Journals.
1994- present	Member of the planning committee of the Annual Industrial Pharmaceutical Research and Development (June Land O' Lakes) Pharmaceutical Conference.
1997	Chair of the June '97 Land O' Lakes Pharmaceutical Conference on **"Challenges and Prospects in the Design and Development of Oral Controlled Release Products"**, June 2-6, 1997, Devil's Head Lodge-Merrimac, WI.
2002-2003	Scientific Advisory Board member of the conference on **Peptide and Protein Formulation Strategies for Drug Delivery and Development** organized by the Institute of International Research.
2003	Chair, Pre-conference Symposium on **"Identifying Opportunities and Overcoming Challenges in Oral Protein and Peptide Delivery"** of the 2nd IIR *Protein & Peptide Formulation Strategies for Drug Development and Delivery*, March 31- April 2, 2003, Boston, MA.
2003	Invited Theme Issue Editor **"Advances in Lipid-Based Drug Solubilization and Targeting"** *Adv. Drug Del. Rev*. 56(9) 7 May, 2004.
2006 - 2008	Chair, Lipid-Based Drug Delivery Systems Focus Group of AAPS.
2006	Co-Chair, 2006 BIO Entrepreneurial Boot Camp for Chief Scientific Officers and Academic Researchers, April 8-9, Chicago, Illinois.
2007	Co-Chair, AAPS Workshop **"Effective Utilization of Lipid-Based Systems for Enhancing the Delivery of Poorly Soluble Drugs: Physicochemical, Biopharmaceutical and Product Development Considerations",** March 5-6, 2007, Bethesda, MD.
2007	Co-chair, iiBIG Conference **"New Directions for Drug Delivery",** October 29-30, 2007, Las Vegas, NV.
2007-2009	Coordinator and Professor in-charge, short course **"Nanoparticles: Applications in Drug Formulation and Delivery"**, Univ.of Wisconsin, Extension Services in Pharmacy.
2008 - 2009	Past Chair, **AAPS Lipid-Based Drug Delivery Systems** Focus Group
2009 - 2011	Chair, **AAPS Nanotechnology Focus Group**.
2010	Elected **Fellow of the American Association of Pharmaceutical Scientists** (AAPS)
2009-present	Editorial Advisory Board Member, **Recent Patents in Drug Delivery and Formulation**, Bentham Science Publishers.
2011	Chair, AAPS Drug Delivery Workshop **"Emerging Oral Delivery Strategies and Technologies to Enable Biopharmaceutical Performance of BCS II, III and IV Molecules**", April 14-15, 2011, Baltimore, MD.

2011	Co-Chair, **4th Annual Nanotechnology Symposium**, Sullivan University, College of Pharmacy, September 23-24, 2011, Louisville, KY.
2012	Chair, 47th AAPS Arden House Conference **"Nanoscience in Pharmaceuticals: Translating Fundamental Understanding to Practical Application in Drug and Device Development**", March 11-14, 2012, The Thayer Hotel, West Point, NY.
11/2012-11/2013	Vice Chair, AAPS Formulation Design and Development (FDD) Section of AAPS.
11/2013-11/2014	Chair-Elect, AAPS Formulation Design and Development (FDD) Section of AAPS.
2013	Chair, Program Committee, **Drug Discovery and Development Track, BIO2013** International Convention, April 22-25, 2013, Chicago, IL.
2012-present	Advisory Committee Member, Nanotechnology Employment, Education and Economic Development, Oakton Community College, Oakton, IL.
2013	Organizer and Co-Chair, Sort Course on **"Quality Control Aspects of Nanoparticulate Dugs: Manufacturing, Characterization and Regulatory Considerations",** Nov. 10, 2013, 2013 AAPS Annual Meeting, San Antonio, TX.
2013	Chair, **3rd International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems (Pharmaceutica-2013),** OMICS Group, April 8-10, 2013, Northbrook, IL.
2014	Chair, **4th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems (Pharmaceutica-2014),** OMICS Group, March 24-26, 2014, San Antonio, TX.
11/2014-10/2015	Chair, Formulation Design and Development (FDD) Section of AAPS.
7/28/2015	2016 AAPS Annual Meeting Jamboree Enterprise Award Recipient.
8/2015 – 4/2021	Associate Editor and Editor, AAPS Open Journal.
10/26/2015	Formulation Design and Development (FDD) Section Chair and Leadership Award, American Association of Pharmaceutical Scientists (AAPS).
2018	Chair, **12th World Drug Delivery Summit**, Annual America Congress Organization, September 24-26, 2018, Chicago, Illinois.
2021	**Recipient of the 2021 International Pharmaceutical Excipients Council (IPEC) Foundation Henk de Jong Industrial Research Award** for outstanding achievements in Excipient Innovation and Research.

Professional Organizations

Past member of the American Biophysical Society, American Association for Cancer Research, American Association for the Advancement of Science and the New York Academy of Sciences, American Chemical Society and the Controlled Release Society. Active member of the American Association of Pharmaceutical Scientists and BIO/iBIO.

Languages

Native is Greek, fluent in English, some knowledge of French.

Recreational Activities

Strong interest in Byzantine art and music. Enjoys mount hiking, bicycling and sports both as spectator and participant.

References Available upon request.

PUBLICATIONS, PRESENTATIONS AND PATENTS

Original Research Papers

1. Panayiotis P. Constantinides and Joseph M. Steim (1985) " **Physical Properties of Fatty Acyl-CoAs: Critical Micelle Concentrations, Micellar Size and Shape** " *J. Biol. Chem*. 260, 7573 - 7580.

2. Panayiotis P. Constantinides and Joseph M. Steim (1986), " **Solubility of Palmitoyl-CoA in Acyltransferase-Assay Buffers Containing Magnesium Ions**" *Arch. Biochem. Biophys*. 250, 267 - 270.

3. Panayiotis P. Constantinides and Joseph M. Steim (1988) " **Micellization of Fatty Acyl CoA Mixtures and Its Relevance to the Fatty Acyl Selectivity of Acyltransferases**" *Arch. Biochem. Biophys.* 261, 430 - 436.

4. Chris A. Pritsos, Panayiotis P. Constantinides, Thomas R. Tritton, David C. Heimbrook and Alan C. Sartorelli (1985) " **Use of High Performance Liquid Chromatography to Detect Hydroxyl and Superoxide Radicals Generated from Mitomycin C** " *Anal. Biochem*. 150, 294 - 299.

5. Panayiotis P. Constantinides, Naoyoshi Inouchi, Thomas R. Tritton, Alan C. Sartorelli, and Julian M. Sturtevant (1986) " **A Scanning Calorimetric Study of the Interaction of Anthracyclines with Neutral and Acidic Phospholipids Alone and in Binary Mixtures** " *J. Biol. Chem.* 261, 10196 - 10203.

6. Panayiotis P. Constantinides, Thomas R. Tritton, and Alan C. Sartorelli (1988), "**Interaction of Adriamycin with Single and Multibilayer Dipalmitoylphosphatidylcholine Vesicles: Spin-labelling and Calorimetric Study** " *J. Liposome Res.* 1, 35 - 62.

7. Robert Dreyer, Edward Hawrot, Alan C. Sartorelli and Panayiotis P. Constantinides (1988) " **Sedimentation Field Flow Fractionation of Fused Unilamellar Vesicles: Comparison with Electron Microscopy and Gel Filtration** " *Anal. Biochem.* 175, 433 - 441.

8. Panayiotis P. Constantinides, Naoyoshi Inouchi, Alan C. Sartorelli and Julian M. Sturtevant (1989) " **Interaction of Adriamycin and N-Trifluoroacetyladriamycin-14-valerate with Cardiolipin-Containing Lipid Bilayers** " *J. Liposome Res.* 1, 245 - 260.

9. Panayiotis P. Constantinides, Lily Ghosaini, Naoyoshi Inouchi, Shinichi Kitamura, Ramakrishnan Seshadri, Mervyn Israel, Alan C. Sartorelli and Julian M. Sturtevant (1989) " **Interaction of N-Alkylanthracyclines with Lipid Bilayers: Correlations Between Partition Coefficients, Lipid Phase Distributions and Thermotropic Behavior** " *Chem. Phys. Lipids* 51, 105 - 118.

10. Panayiotis P. Constantinides, Yan Yan Wang, Thomas G. Burke, and Thomas R. Tritton (1990) "**Tranverse Location of Anthracyclines in Lipid Bilayers: Paramagnetic Quenching Studies** " *Biophys. Chem.* 35, 259-264.

11. Bruce Babbitt, Lisa Burtis, Patrick Dentinger, Panayiotis Constantinides, Larry Hillis, Barbara McGirl and Leaf Huang (1993) **"Contact-Dependent, Immunecomplex-Mediated Lysis of Hapten-Sensitized Liposomes"** *Bioconjugate Chem.* 4, 199-205.

12. Panayiotis P. Constantinides, Jean-Paul Scalart, Cindy Lancaster, Joseph Marcello, Gary Marks, Harma Ellens and Philip Smith (1994) **"Formulation and Intestinal Absorption Enhancement Evaluation of Water-in-Oil Microemulsions Containing Medium-Chain Glycerides "** *Pharm. Research* 11 (10), 1385-1390.

13. Panayiotis P. Constantinides, Cindy M. Lancaster, Joseph Marcello, D. Chiossone, Donald Orner, Ismael Hidalgo, Philip L. Smith, Ani B. Sarkahian, Seang H. Yiv and Albert J. Owen (1995) **"Enhanced Intestinal Absorption of an RGD Peptide from Water-in-Oil Microemulsions of Different Composition and Particle Size"**, J. Control. Rel., 34, 109-116.

14. Panayiotis P. Constantinides and Seang H. Yiv (1995) **"Particle Size Determination of Phase-Inverted Water-in-Oil Microemulsions Under Different Dilution and Storage Conditions",** *Int. J. Pharm.* 115, 225-234.

15. Panayiotis P. Constantinides, Gus Welzel, Harma Ellens, Philip L. Smith, Sandy Sturgis, Seang H. Yiv and Albert J. Owen (1996) "**Water-in-oil Microemulsions Containing Medium-Chain Fatty Acid/Salts : Formulation and Intestinal Absorption Enhancement Evaluation"** *Pharm. Res*. 13, 210-215.

16. Hung-Yuan Cheng, Cynthia S. Randall, Walter W. Holl, Panayiotis P. Constantinides, Tian-Li Yue and Giora Z. Feuerstein (1996)**"Carvedilol-Liposome Interaction: Evidence for Strong Association with the Hydrophobic Region of Bilayers",** *Biochim. Biophys. Acta* 1284, 20-28.

17. Panayiotis P. Constantinides and Jean-Paul Scalart (1997) **" Formulation and Physical Characterization of Water-in-Oil Microemulsions Containing Long- versus Medium-Chain Glycerides "** *Int. J. Pharm.* 158: 57-68 (1997).

18. Panayiotis P. Constantinides, Karel Lambert, Alexander K. Tustian, Wenwen Ma, Brian Schneider, Salima Lalji, Bryan Wentzel, Dean Kessler, Dilip Worah and Steven C. Quay (2000) **"Formulation Development and Antitumor Activity Evaluation of a Filter-Sterilizable Emulsion of Paclitaxel",** *Pharm. Res*. **17** : 175-182.

19. Pavel Gershkovich, Jerald Darlington, Panayiotis P. Constantinides and Kishor M. Wasan (2009) **Inhibition of Intestinal Absorption of Cholesterol by Surface-Modified Nanostructured Aluminosilicate (NSAS) Compounds.** *J. Pharm. Sci*.**98**: 2390-2400.

20. Olena Sivak, Jerald Darlington, Pavel Gershkovich, Panayiotis P. Constantinides and Kishor M. Wasan (2009) **Protonated Nanostructured Aluminosilicate Reduces Plasma Cholesterol Concentrations and Atherosclerotic Lesions in Apolipoprotein Deficient Mice Fed a High Cholesterol and High Fat Diet**, *Lipids Health Dis*. Jul 28; 8 (1) : 30, online publication.

21. G. Xie, T. Nie, G.C. Mackenzie, Y. Sun, L. Huang, N. Ouyang, N. Alston, O.T. Murray, P.P. Constantinides, L. Kopelovich and B. Rigas (2011), **The Metabolism and Pharmacokinetics of Phospho-sulindac (OXT-328) and the Effect of Difluoromethylornithine**, *Br. J. Pharmacol.* 2011 Sep 28. Doi:10.1111/j.1476-5381.2011.01705.x [Epub]. .

22. George Mattheolabakis, Ting Nie, Panayiotis P. Constantinides and Basil Rigas (2012), **Sterically Stabilized Liposomes Incorporating the Novel Anticancer Agent Phospho-Iburpofen (MDC-917): Preparation, Characterization and In Vitro/In Vivo Evaluation,** *Pharm. Res*. 29: 1435 - 1443.

23. Chi C. Wong, Ka-Wing Cheng, Gang Xie, Dingying Zhou, Cai-Hua Zhu, Panayiotis P. Constantinides and Basil Rigas (2012), **Carboxyesterases 1 and 2 Hydrolyse Phospho-NSAIDs to Their Pharmacological Activity**, *J. Pharmacol.. Exp. Ther*. 340:422-432.

24. Ting Nie, Chi C. Wong, Niche Alston, Patrick Aro, Panayiotis P. Constantinides and Basil Rigas (2012**), Phospho-Ibuprofen (MDC-917) Incorporated in Nanocarriers: Anticancer Activity In Vitro and In Vivo**, *Br.J.Pharmacol*. 166: 991-1001.

25. K.A.Wing Cheng, Georgios Mattheolabakis, C.C.Wong, Nengtai Ouyang, Liqun Huang, Panayiotis P. Constantinides and Basil Rigas (2012) **Topical Phosphor-Sulindac (OXT-328) Is Effective in the Treatment of Non-Melanoma Skin Cancer**, *Int. J. Oncol.* 41: 1199-1203.

26. C. Zhu, K.W.Cheng, N. Ouyang, L. Huang, Y. Sun, P.P.Constantinides and B. Rigas (2012), **Phosphosulindac (OXT-328) Selectively Targets Breast Cancer Stem Cells In vitro and in Human Breast Cancer Xenografts**. *Stem Cells*, May 31, 2012, doi: 10.1002/stem.1139 [E-pub].

27. G.Xie, C.C.Wong, K.W.Cheng, L. Huang, P.P.Constantinides and B. Rigas (2012) **Regioselective Oxidation of Phospho-NSAIDs by Human Cytochrome P450 and Flavin Monooxygenase Isoforms : Implications for Their Pharmacokinetic Properties and Safety**, *Br. J. Pharmacol.* 167: 222-232.

28. G.Xie, C.C.Wong, K.W.Cheng, L. Huang, P.P. Constantinides and B. Rigas (2012) **In Vitro and In Vivo Studies of Phospho-Aspirin (MDC-22)**, *Pharm Res* 29 : 3292-3301.

29. R. Zhu, K.W.Cheng, G. Makenzie, L. Huang, Y. Sun, G.Xie, K.Vrankova, P.P.Constantinides and B. Rigas (2012**) Phospho-Sulindac (OXT-328) Inhibits the Growth of Human Lung Cancer Xenografts in Mice : Enhanced Efficacy and Mitochondria Targeting by its Formulation in Solid Lipid Nanoparticles,** *Pharm Res*. 29 : 3090-3101.

30. George Mattheolabakis, Chi C. Wang, Yu Sun, Carol Ann Amelia, Robert Richards, Panayiotis P. Constantinides and Basil Rigas (2014) **Pegylation improves the pharmacokinetics and bioavailability of small-molecule drugs hydrolysable by esterases : A study of phosphor-ibuprofen,** *J. Pharmacol. Exp. Ther.* 351: 61-66.

Commentaries

1. Panayiotis P. Constantinides, Subhashis Chakraborty and Dali Shukla, **Considerations and Recommendations on Traditional and Non-traditional Uses of Excipients in Oral Drug Products**. AAPS Open (2016) 2(1), 1-6 DOI 10.1186/s41120-016-0004-3.

2. Panayiotis P. Constantinides, **Join the Dialogue: Do We Need an Excipient Classification System Based on Their Traditional and Non-Traditional Uses in Drug Products?** AAPS Blog Article posted on July 14, 2016.

Review Articles/Book Chapters/Book Reviews

1. Panayiotis P. Constantinides (1995) "**Lipid Microemulsions for Improving Drug Dissolution and Oral Absorption: Physical and Biopharmaceutical Aspects**", *Pharm. Res*. **12:** 1561-1572.

2. Panayiotis P. Constantinides and Ron Liu (2000) **"Micellization and Drug Solubility Enhancement "** in *Water-Insoluble Drug Formulation* (Liu, R. Ed.), Chapter 9, pp. 213-277, Interpharm Press Inc., Denver, Colorado.

3. Panayiotis P. Constantinides (2000) **"Self-Emulsifying Drug Delivery Formulations in the 21st Century: Challenges and Opportunities"** in *Controlled Drug Delivery: Designing Technologies for the Future* (K. Park and R.J. Mrsny, Eds), ACS Symp. Series 752, 284 – 296.

4. Panayiotis P. Constantinides and Kishor M. Wasan (2004) **"Advances in Lipid-Based Drug Solubilization and Targeting"**, in *Advances in Lipid-Based Drug Solubilization and Targeting* (Constantinides, P.P. and Wasan, K. Eds), *Adv. Drug Del. Rev*., **56** (9) pp. 1239-1240.

5. Panayiotis P. Constantinides, Alex Tustian and Dean Kessler (2004) **"Tocol Emulsions for Drug Solubilization and Parenteral Delivery"** in *Advances in Lipid-Based Drug Solubilization and Targeting* (Constantinides, P.P. and Wasan, K. Eds), *Adv. Drug Del. Rev*., **56** (9) pp. 1243-1255.

6. Panayiotis P. Constantinides, Jihong Han and Stanley S. Davis (2006**) "Advances in the Use of Tocols as Drug Delivery Vehicles"**, *Pharm. Res*. 23 (2) 243-255.

7. Panayiotis P. Constantinides and Kishor M. Wasan (2007) **"Lipid Formulation Strategies for Enhancing Intestinal Transport and Absorption of P-glycoprotein (P-gp) Substrate Drugs: In vitro/In vivo Case Studies",** *J. Pharm. Sci*. **96** (2) 235-248.

8. Panayiotis P. Constantinides, Mahesh Chaubal and Robert Shorr (2008) **"Advances in Lipid Nanodispersions for Parenteral Drug Delivery and Targeting",** theme issue on *Lipid-Based Systems for Enhanced Delivery of Poorly Soluble Drugs* (Christopher J.H. Porter, Kishor M. Wasan and Panayiotis P. Constantinides, Editors) *Adv. Drug Del. Rev*. **60**, pp. 757-767.

9. Panayiotis P. Constantinides (Book Review). **Role of Lipid Excipients in Modifying Oral and Parenteral Drug Delivery**. Kishor M. Wasan (Ed.), Wiley-Interscience 2007. *Drug Dev. Ind. Pharm*. (2008) 34(5): 558.

10. Navdeep Kaur, Ashwini Gadre, Panayiotis P. Constantinides and Yashwant Pathak (2010) **Nanomedicine: Trends and Perspectives on Technologies and Products**, in *Advances in Nanotechnology and Applications*, Vol. 2, Center for Nanotechnology, Education, Research and Applications, Sullivan University, College of Pharmacy, Louisville, KY.

11. Panayiotis P. Constantinides (2011) **Advances in Nanotechnology and Commercialization Perspectives**, Foreword in *Advances in Nanotechnology and Applications*, Vol. 3, Center for Nanotechnology, Education, Research and Applications, Sullivan University, College of Pharmacy, Louisville, KY.

12. Roy Haskell, Panayiotis P. Constantinides and Duxin Sun **"Perspectives in Pharmaceutical Nanotechnology"**, cover article, AAPS News Magazine January 2012.

13. George Mattheolabakis, Basil Rigas and Panayiotis P. Constantinides (2012), **Nanodelivery Strategies in Cancer Chemotherapy: Biological Rationale and Pharmaceutical Perspectives**, *Nanomedicine* 7 (10): 1577-1590.

14. Liu Changxiao, Panayiotis P. Constantinides, Yazhuo, Li (2014) **R&D in Drug Innovation: Reflections from the 2013 Bioeconomy Conference in China, Lessons Learned and Future Perspectives**, *Acta Pharmaceutica Sinica B*, 4 (2) : 112-119.

Interview Publications

1. **Oral Drug Delivery Technologies: Tackling Clinical and Commercial Challenges"**, Deborah Erickson, *Fierce Pharma*, March 2012.

2. **Nanoparticles : A Look Forward** technical retrospective, a look ahead at challenges and opportunities for the development of nanoparticle drug delivery systems, Amy Ritter (Moderator), *BioPharm International*, 25 (6) : 28 (2012).

3. **Nanoformulations**, Q&A session with Biopharmaceutical & Drug Delivery Consulting, LLC moderated by Amy Ritter, *Pharm Tech*, July 2012.

4. **IPEC Foundation Recognizes Panayiotis P. Constantinides**, a recipient of the 2021 IPEC Foundation Henk de Jong Industrial Research Achievement for Excipient Technology Award. Published in the *February 2022 Issue of IPEC Americas Insider*.

Professional Development Articles

1. Panayiotis P. Constantinides, **Building a Consulting Business : A Practitioner's Perspective**, AAPS News Magazine May 2016, pp. 31-34.

Abstracts/Poster Presentations

1. Panayiotis P. Constantinides, Naoyoshi Inouchi, Thomas R. Tritton, Alan C. Sarorelli, and Julian M. Sturtevant (1986) **"Comparative Study of the Interaction of Anthracyclines with Lipid Bilayers Using High Sensitivity Differential Scanning Calorimetry "** ***Biophysical J. 49, 511.*** *Presented at the 30th Annual Biophysical Society Meeting, February 9-13, 1986, San Francisco, California.*

2. Panayiotis P. Constantinides, Naoyoshi Inouchi, Alan C. Sartorelli, and Julian M. Sturtevant (1986) **" Interaction of Anthracyclines with Cardiolipin-Containing Neutral and Acidic Liposomes Using High Sensitivity Differential Scanning Calorimetry "** *Delivered at the 41st Calorimetry Conference, August 17-22, 1986, Somerset, New Jersey, Abstract No. 79.*

3. Panayiotis P. Constantinides, Lily Ghosaini, Naoyoshi Inouchi, Shinichi Kitamura, Ramakrishnan Seshadri, Mervyn Israel, Alan Sartorelli, and Julian M. Sturtevant (1987) **" Interaction of N-Alkylanthracyclines with Lipid Bilayers Using High Sensitivity Differential Scanning Calorimetry "** ***Biophysical J. 51, 239.*** *Delivered at the 31st Annual Biophysical Society Meeting, February 22-26, 1987, New Orleans, Louisiana.*

4. Panayiotis P. Constantinides, Jean-Paul Scalart, C. Lancaster, J. Marcello, G. Marks, H. Ellens and P. Smith (1993), **"Water-in-Oil Microemulsions Containing Medium-Chain Glycerides: Formulation and Absorption Enhancement Evaluation in the Rat"** ***Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 20: 184-185.*** Delivered at the 20th International Symposium of the Controlled Release Society, July 25-28, 1993, Washington, DC.

5. Panayiotis P. Constantinides, Jean-Paul Scalart, Cindy Lancaster, Joseph Marcello, Gary Marks, Harma Ellens, P. Smith, Andrew Nichols, Janice Vasko, Paul Koster, Gerald Rhodes, C.

Miller-Stein, Richard Simpson, Fadia Ali and James Samanen (1994) **"Enhancement of RGD Peptide Oral Activity with a Water-in-Oil Microemulsion"**. Presented at the Gordon Conference on Chemistry and Biology of Peptides, February 13-17, 1994 , Ventura, CA.

6. Panayiotis P. Constantinides, Patrick Dentinger and Leaf Huang (1994), **"Incorporation of Lipophilic Drugs into Liposomes from Lipid : Ethanol Admixtures and Comparisons with Conventional Liposomes"**. *Proceed. Intern.. Symp. Control. Rel. Bioact. Mater. 21, 501-50,* June 27-30, 1994, Nice, France.

7. Panayiotis P. Constantinides, Cindy Lancaster, J. Marcello, D. Chiossone, D. Orner, I. Hidalgo, A. Sarkahian, S. H. Yiv, A. B. Owen and P. L. Smith, (1994) **"Oral Absorption Enhancement of an RGD Peptide from Water-in-Oil Microemulsions of Different Composition and Particle Size"**. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 21, 62-63,* June 27-30, 1994, Nice, France.

8. Panayiotis P. Constantinides and Seang H. Yiv, (1994), **" Particle Size Determination of Phase-Inverted Water-in-Oil Microemulsions Under Different Dilution and Storage Conditions"** *Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 21,766-767.* June 27-30,1994, Nice, France.

9. Gary. J. Marks, Gus. Welzel, Philip. L. Smith, Panayiotis .P. Constantinides and H. Ellens, (1995), **"Bioavailability Enhancement of Hydrophilic Molecules by Medium-Chain Glycerides in the Rat".** Presented at the *Intern. Symp. Control. Rel. Bioact. Mater 22*, July 30-August 4, 1995, Seattle, WA.

10. Panayiotis P. Constantinides, Gus Welzel, Harma Ellens, Philip L. Smith, Sandy Sturgis, Seang H. Yiv and Albert J. Owen (1996) "**Water-in-oil Microemulsions Containing Medium-Chain Fatty Acid/Salts : Formulation and Intestinal Absorption Enhancement Evaluation ".** Presented at the annual meeting of the American Association of Pharmaceutical Scientists, October 27-31, 1996, Seattle, WA.

11. Panayiotis P. Constantinides, Karel Lambert, Alexander K. Tustian, Wenwen Ma, Brian Schneider, Salima Lalji, Bryan Wentzel, Dean Kessler, Dilip Worah and Steven C. Quay, **"Reduced Toxicity and Improved Efficacy of Paclitaxel Incorporated in Oil-in-Water Emulsions"** presented at the AAPS Annual Meeting, November 15-19, 1998, San Francisco, California.

12. Panayiotis P. Constantinides, Dean Kessler, Alexander Tustian, Karel Lambert and Eric A. Rowinsky **" Antitumor Activity of QW8184, an Injectable Paclitaxel Emulsion, and Taxol® in the B16 Melanoma and IGROV-1 Ovarian Tumor Xenograft Models in Mice"** presented at the AACR-NCI-EORTC International Conference on *"Molecular Targets and Cancer Therapeutics: Discovery, Development and Clinical Validation"*, November 16-19 , 1999, Washington, DC.

13. Eun-Hyun Jang, Likan Liang and Panayiotis P. Constantinides, **"Factors Controlling the *In Vitro* Release of Rhodamine-Dextran from Polymerized Liposomes in Simulated Intestinal Fluid",** 29th Annual Meeting and Exposition of the Controlled Release Society, July 20-25, 2002, Seoul, Korea.

14. Panayiotis P. Constantinides, Likan Liang, Eun-Hyan Jang, David J. Fast, Liangxiu He, Lanlan Li and Kayode Opeifa **"Enhanced Intestinal Absorption of LHRH and Leuprolide In The**

Rat from Lipid Polymer Micelles", 29th Annual Meeting and Exposition of the Controlled Release Society, July 20-25, 2002, Seoul, Korea.

15. Panayiotis P. Constantinides, Likan Liang, David J. Fast, Sumeet Dagar, Liangxiu He, Lanlan Li and Kayode Opeifa **"Bioavailability Enhancement of Leuprolide Upon Intraduodenal Administration in Dogs from Lipid Polymer Micelles (LPM™)"**, 2002 Annual AAPS Meeting and Exposition, November 10 – 14, 2002, Toronto, Canada.

16. Dave J. Fast, Reena Patil, Kevin Bosman and Panayiotis P. Constantinides, **"Lipid Polymer Emulsions (LPE™) Incorporating P-glycoprotein Inhibitors Enhance the Intestinal Absorption of Paclitaxel"**, 2002 Annual AAPS Meeting and Exposition, November 10 – 14, 2002, Toronto, Canada.

17. Jerry W. Darlington and Panayiotis P. Constantinides. **Virus Inactivation by Nanobentonite in Suspended Cells and on Hard Surfaces.** BIO 2007 International Convention, Innovation Corridor Poster Session, May 6-9, 2007, Boston, MA

18. Olena Sivak, Pavel Gershkovich, Jerald Darlington, Panayiotis P. Constantinides and Kishor M. Wasan. **Potential Anti-hyperlipidemic Activity of Nanoscale Aluminosilicate (NSAS) in Rabbits**. 2008 AAPS Annual Meeting and Exposition, November 16-20, 2008 Atlanta, GA

19. Pavel Gershkovich, Olena Sivak, Jerald Darlington, Panayiotis P. Constantinides and Kishor M. Wasan. **Inhibition of Intestinal Absorption of Cholesterol by Novel Aluminosilicates (NSAS) compounds.** 2008 AAPS Annual Meeting and Exposition, November 16-20, 2008 Atlanta, GA.

20. Pavel Gershkovich, Jerald Darlington, Panayiotis P. Constantinides and Kishor M. Wasan. **Protonated Nanoscale Aluminosilicate (NSAS) Reduces Plasma Cholesterol Concentrations and Atherosclerotic Lesion Formation in Apolipoprotein E (ApoE)-Deficient Mice".** 2009 AAPS Annual Meeting and Exposition, November 8-12, 2009, Los Angeles, CA.

Invited Talks

1. Panayiotis P. Constantinides, Jean-Paul Scalart, Joe Marcello, Richard Kirsh and Phil Smith (1991) **"Optimization and Utilization of Microemulsion Delivery Systems for Oral Administration of Peptidergic Drugs"** *2nd Annual SmithKline Beecham Drug Delivery Workshop, June 25-26, 1991, Harlow, UK.*

2. Panayiotis P. Constantinides and Jean-Paul Scalart **"Self-Emulsifying Water-in-Oil Microemulsions in Drug Delivery: Formulation and Physical Characterization"** *First International Conference on Pharmaceutical Science and Technology and the ACS Fine Particle Society, August 24-28, 1993, Chicago, Illinois.*

3. Panayiotis P. Constantinides **"Self-Emulsifying Water-in-Oil Microemulsions in Drug Delivery: Formulation and Oral Absorption Enhancement Evaluation"** *"Emulsion Day" symposium, SmithKline Beecham Consumer Brands, Nov. 1, 1993, Weybridge, UK.*

4. Panayiotis P. Constantinides, **"Intestinal Absorption Enhancement Using Microemulsion Formulations"**, *Second International Symposium on Pharmaceutical Sciences and Technology of the Fine Particle Society, July 25-28, 1994, E. Brunswick, NJ.*

5. Panayiotis P. Constantinides and Seang H. Yiv, "**Particle Size Measurements of Phase Inverted Water-in-Oil Microemulsions"**, lead lecture at the *Second International Symposium on Pharmaceutical Sciences and Technology of the Fine Particle Society, July 25-28, 1994, E. Brunswick, NJ.*

6. Panayiotis P. Constantinides, Patrick Dentinger and Leaf Huang, **"Admixture Liposomes Solubilizing Lipophilic Drugs: Characterization and Potential Applications",** *Second International Conference on Pharmaceutical Sciences and Technology of the Fine Particle Society, July 25-28, 1994, E. Brunswick, NJ.*

7. Panayiotis P. Constantinides, **"Formulation Strategies to Improve the Oral Absorption of Poorly Absorbed Drugs",** invited quest seminar at the *Philadelphia College of Textiles and Science, October 20, 1994 Philadelphia, PA.*

8. Panayiotis P. Constantinides, **"Formulation Design/Development Considerations of Multiphase Systems for Parenteral and Oral Drug Delivery"**, invited speaker and a member of the organizing committee of the 37th Annual International Industrial Pharmaceutical Research and Development Land O' Lakes Conference on "Multiphase Systems for Parenteral and Oral Drug Delivery: Physical and Biopharmaceutical Aspects", June 5-9, 1995, Merrimac, Wisconsin.

9. Panayiotis P. Constantinides, **"Water-in-oil Microemulsions for Oral Delivery of Drugs/Peptides with High Aqueous Solubility and Low Membrane Permeability**", delivered at the American Chemical Society's "Conference on Formulations and Drug Delivery", October 10-13, 1995, Boston, Massachusetts.

10. Panayiotis P. Constantinides, **"Lipid Microemulsions as a Novel Dosage Form for Poorly Absorbed Drugs",** delivered at the Food and Drug Administration Agency, October 18, 1996, Rockville, Maryland.

11. Panayiotis P. Constantinides, "**Drug Development Aspects with Water-in-Oil Microemulsions for Oral Delivery of Poorly Absorbed Water-Soluble Drugs/Peptides ",** delivered at the symposium on *Lipid-based systems for oral drug delivery: Physiological, Mechanistic and Product Development Perspectives,* Nov. 3, 1997 AAPS Annual Meeting, Boston, MA.

12. Panayiotis P. Constantinides, **"Physical and Biopharmaceutical Aspects of Self-Emuslifying Microemulsion Systems for Oral Drug Delivery",** delivered at Pharmacia & Upjohn (Pharmaceutical Development), February 2, 1998, Kalamazoo, Michigan.

13. Panayiotis P. Constantinides, **"Challenges and Opportunities in the Use of Self-Emulsifying Drug Delivery Systems for Oral Drug Delivery and Intestinal Absoprtion Enhancement",** delivered on March 11, 1999, University of Washington School of Pharmacy, Department of Pharmaceutics and Medicinal Chemistry.

14. Panayiotis P. Constantinides, **"Self-Emulsifying Drug Delivery Systems in the 21st Century : Challenges and Opportunities"**, delivered at the ACS Symposium on ***"Drug Delivery in the 21st Century"***, March 21-23, 1999, Anaheim, California.

15. Panayiotis P. Constantinides, "**Product Development Opportunities with Alternative Drug Delivery Systems for Marketed Chemotherapeutics",** delivered at the IBC Conference on

"Drug Delivery Systems: Strategies for Competitive Advantage", May 24-25, 1999, Washington, DC.

16. Panayiotis P. Constantinides, **"Lipid Microemulsions in Drug Solubilization and Delivery"**, delivered at the ACS Symposium *"Microemulsions: Properties and Applications"*, August 20 – 21, 2000, Washington, DC.

17. Panayiotis P. Constantinides, Karel J. Lambert, Alex K. Tustian, Salima Lalji and Dean Kessler, **"Stable and Efficacious Filter Sterilizable Tocol Microemulsions- A Case Study with Paclitaxel"**, invited paper at the *Pharmaceuticals 2000* virtual conference (internet based), November 6-10, 2000.

18. Michael S. Rosen and Panayiotis P. Constantinides, " **Oral Drug/Peptide : Commercial and Technological Challenges**", delivered at the 6th US-Japan Symposium on Drug Delivery Systems, Dec. 16-21, 2001, Maui, Hawaii.

19. Panayiotis P. Constantinides, **"Emulsion and Micellar Nanoparticles for Oral Drug/ Peptide Delivery"**, delivered at the Gordon Research Conference on Drug Carriers in Medicine & Biology, Feb. 24 – March 1, 2002, Ventura, California.

20. Panayiotis P. Constantinides **"Lipid Microemulsions and Micellar Nanoparticles for Oral Drug/Peptide Delivery"** invited talk at the Particles 2002 International Conference: **Medical/Biochemical, Diagnostic, Pharmaceutical, and Drug Delivery Applications of Particle Technology**, April 20-23, 2002, Orlando, Florida.

21. Panayiotis P. Constantinides **"Lipid Polymer Micelles and Emulsions for Improving Oral Drug/Peptide Absorption"**, presented at the 19th Technology Transfer Forum of the Technology Catalysts, Inc., May 12-14, 2002, Reston, Virginia.

22. Panayiotis P. Constantinides, **"Development of Oral Peptide Formulations Using Lipid-Based Microemulsion and Micellar Delivery Systems"**, delivered at the *Protein & Peptide Formulation Strategies for Drug Development and Delivery* ,pre-conference workshop on Strategies for Formulating Macromolecules for Oral Delivery, August 19-20, 2002, San Francisco, California, presented by the Institute for International Research and Drug Delivery Partnerships.

23. Panayiotis P. Constantinides, Charles Conover, Steven J. Prestrelski and Thomas Tice, panel discussion on **"Managing Intellectual Property During Drug Delivery Partnerships"**, *Protein & Peptide Formulation Strategies for Drug Development and Delivery*, August 19-20, 2002, San Francisco, California.

24. David J. Fast, Reena Patil, Kevin Bosman, Lori-Pokorsky Loy and Panayiotis P. Constantinides, **"Enhancement of Paclitaxel Transport and Intestinal Absorption Using Lipid Polymer Emulsions (LPE™) Incorporating P-glycoprotein Inhibitors"**, *International Symposium on Tumor Targeted Delivery Systems* sponsored by CRS/NCI, September 23-25, 2002, Bethesda, Maryland.

25. Panayiotis P. Constantinides, **"Identifying Opportunities and Overcoming Challenges in Oral Protein and Peptide Delivery"** Pre-conference Symposium chair and introductory talk, 2nd Intern.. Institute of Research (IIR) *Protein & Peptide Formulation Strategies for Drug Development and Delivery*, March 31- April 2, 2003, Boston, MA.

26. Panayiotis P. Constantinides, **"Dispersed Systems in Oral Peptide/Protein Delivery : Microemulsion and Micellar Systems"** invited talk at the Pre-conference Symposium of the 2nd IIR *Protein & Peptide Formulation Strategies for Drug Development and Delivery,* March 31- April 2, 2003, Boston, MA

27. Panayiotis P. Constantinides, **"Formulation Strategies to Overcome Drug Absorption Barriers Due to Intestinal Efflux Pumps"**, invited talk at the IIR's *Oral Drug Delivery Conference*, June 23-24, 2003, Boston, MA.

28. Panayiotis P. Constantinides, **"Overcoming Biological Barriers to the Oral Absorption of Small Molecule and Macromolecular Drugs Using Lipid-Based Systems",** invited talk at the University of Cyprus, March 31, 2004, Nicosia, Cyprus.

29. Panayiotis P. Constantinides, **"Intravascular and Oral Delivery of the Chemotherapeutic Drug Paclitaxel Using Microemulsifying Lipid Systems ",** invited talk at Hebrew University, April 14, 2004, Jerusalem, Israel.

30. Panayiotis P. Constantinides, **"Advanced Tocol Emulsions and Lipid Polymer Emulsions for Parenteral and Oral Delivery of Poorly Soluble Drugs"**, invited talk at Eastman Chemical, May 14, 2004, Kingsport, TN.

31. Panayiotis P. Constantinides, **"Case Study : Injectable Drug Products"** invited talk at the 2004 June Land O' Lakes Conference on *"Role of Excipients in Solubility and Bioavailability Enhancement : Current Approaches, Unmet Needs, and Future Directions*", Merrimac, Wisconsin, June 7-11, 2004.

32. Panayiotis P. Constantinides **"Effective Utilization of Lipid-Based Systems to Enhance Solubility and Bioavailability of Small Molecule and Macromolecule Drugs"**, invited talk at ALZA Corporation/Johnson & Johnson, September 24, 2004, Mountain View, CA.

33. Panayiotis P. Constantinides, **"Scientific and Technological Advances of Nanotechnology""**, moderator and panelist, session on *"Nanotechnology and Drug Therapy"* of the 2004 Marketplace Meeting sponsored by the Illinois Biotech Industry Organization (IBIO), October 25-26, 2004, Chicago, Illinois.

34. Panayiotis P. Constantinides, **"Effective Utilization of Oral Lipid Formulations to Overcome Intestinal Drug Transport and Membrane Permeability Barriers"**, invited talk at the Barnett International Conference on "*Lipid-Based Formulations/Drug Delivery"*, September 29-30, 2004, Philadelphia, PA.

35. Panayiotis P. Constantinides, **"Tocol Emulsions for Drug Solubilization and Parenteral Delivery",** invited talk at the symposium *"New Developments in Parenteral Lipid-Based Drug Delivery Systems",* (P. Constantinides and K. Wasan, symposium organizers), 2004 American Association of Pharmaceutical Scientists, Annual Meeting, November 7-11, 2004, Baltimore, MD.

36. Panayiotis P. Constantinides, **"Opportunities and Challenges in the Development of Combination Products of Oral Drugs with P-glycoprotein Limited Absorption**", invited talked at the Parexel Conference on *"Fixed Combination Product Development"*, March 7-8, 2005, San Diego, CA.

37. Panayiotis P. Constantinides, **"Drug Solubillity and Bioavailability Enhancement Using Lipid Emulsion and Micellar Systems",** invited talk at the University of Kentucky, College of Pharmacy, April 1, 2005, Lexington, KT.

38. Panayiotis P. Constantinides, **"Biopharmaceutical and Pharmaceutical Technology Aspects of Combination Drug Development : Oral Drugs with P-glycoprotein Limited Absorption**", invited talked at the Parexel's Conference on *"*Combination Product Development *: Leveraging the Current Scientific, Regulatory and Legal Environment to Gain Regulatory Approval "*, June 9-10, 2005, Brussels, Belgium.

39. Panayiotis P. Constantinides, "**Development of Lipid Formulations for Oral Drugs Exhibiting P-glycoprotein Limited Absorption ",** invited talk at the Chicagoland Pharmaceutical Discussion Group, December, 1, 2005.

40. Panayiotis P. Constantinides, **"Project, Product or Company : A CSO Perspective"**, invited talk at the *BIO 2006 Entrepreneurial Boot Camp for Chief Scientific Officers and Academic Researchers*, Session 4, April 8-9, 2006, Chicago, IL.

41. Panayiotis P. Constantinides, **"Critical Role of Biopharmaceutics in Bridging Product Quality and Product Performance"**, invited talk at the symposium *Challenges and Opportunities on the Critical Path to Lipid-Based Oral Dosage Forms-Assessment of Product Quality, Product Performance and Therapeutic Equivalence"*, Annual AAPS Meeting and Exhibition, October 29-November 2, 2006, San Antonio, TX.

42. Panayiotis P. Constantinides, **"Advances in the Use of Lipid-Based Systems for Parenteral Drug Delivery"**, invited talk at the AAPS Workshop on *Effective Utilization of Lipid-Based Systems to Enhance the Delivery of Poorly Soluble Drug : Physicochemical, Biopharmaceutical and Product Development Considerations* (P.P.Constantinides and C.H. Porter, organizers), March 5-6, 2007, Bethesda, MD.

43. Panayiotis P. Constantinides, **"Overcoming Physicochemical and Biological Barriers to Drug/Peptide Delivery Using Lipid-Based Systems**" invited seminar at the Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada, March 13, 2007.

44. Panayiotis P. Constantinides, **"Project, Product or Company : A CSO Perspective"**, invited talk at the *BIO 2007 The Biotechnology Entrepreneurship Boot Camp*, Session 4, May 6-9, 2007, Boston, MA.

45. Panayiotis P. Constantinides, course coordinator and lead faculty on **"Nanoparticles : Applications in Drug Formulation and Delivery",** University of Wisconsin Extension Services in Pharmacy, May 21-23, 2007, Madison, WI.

46. Panayiotis P. Constantinides, **"Advances and Future Developments in Drug Delivery Nanotechnology"**, invited Workshop A talk, iiBIG conference *on "New Directions for Drug Delivery"*, (co-chaired by Panayiotis P. Constantinides and Mahesh Chaubal), October 29-30, 2007, Las Vegas, NV.

47. Panayiotis P. Constantinides, "**Lipid Formulation Strategies for Enhancing Solubility and Permeability of BCS IV Drugs : *In Vitro/In vivo* Case Studies"**, invited talk at the symposium *BCS IV Drugs : Develop or Discard*, 2007 AAPS Annual Meeting, Nov. 11- 15, 2007, San Diego, CA.

48. Panayiotis P. Constantinides, moderator and speaker in a Workshop on **"Biomedical Nanotechnology: Progressing from Bench to Clinic to Commercialization"**, iBIO IndEx 2008, Feb. 20, 2008, Chicago, IL.

49. Panayiotis P. Constantinides, course coordinator and lead faculty on **"Nanoparticles : Applications in Drug Formulation and Delivery",** University of Wisconsin Extension Services in Pharmacy, May 12-14, 2008, Madison, WI.

50. Panayiotis P. Constantinides **"Oral and Injectible Nanoparticles",** invited talk at the *50th Annual June LOL International Industrial Pharmaceutical R&D Conference* **"Designing Drug Delivery Systems : Past, Present and Future Opportunities for Treating Our Patients"**, June 2-6, 2008, Merrimac, Wisconsin.

51. Panayiotis P. Constantinides, **"Project, Product or Company : A CSO Perspective"**, invited talk at the *BIO 2008 The Biotechnology Entrepreneurship Boot Camp* , Session 4, June 16-17, 2008, San Diego, CA.

52. Panayiotis P. Constantinides **"Diversity and Versatility of Lipids in Enhancing the Delivery of Drugs: Physicochemical and Biopharmaceutical Aspects",** session on *Lipids in Pharmaceutics* (A.Tselepis and P.P. Constantinides, co-chairs), 6th Euro Fed Lipid Congress, Sept. 7-10, 2008, Athens, Greece.

53. Panayiotis P. Constantinides **"Scientific and Technological Advances in Nanoparticles for Drug Formulation and Delivery"** and **"Oral Self-Assembled Lipid Nanostructures in Drug Delivery: Physicochemical and Biopharmaceutical Aspects"**, Nanomedicines 08 Intensive Course and Drug Delivery Workshop, Sept. 12-22, 2008, University of Patras, Greece.

54. Panayiotis P. Constantinides **"Formulation Development Considerations for Liquid-Filled Hard Capsules"**, a symposium on *Emerging Technologies: Liquid Fill Hard Capsule Technology in the Pharmaceutical Industry*, AAPS Annual Meeting, November 16-20, 2008, Atlanta, GA.

55. Panayiotis P. Constantinides, **"Advances in Lipid Nanodispersions and Nanoparticles for Non-Oral Drug Delivery and Targeting"** invited keynote talk at the workshop *"Scientific and Technological Advances in the Use of Lipid-Based Drug Delivery Systems for Bioavailability Enhancement and Tissue Targeting"*, March 9-11, 2009, Baltimore, MD.

56. Panayiotis P. Constantinides **"Product Development Considerations on the Use of Nanoparticles in Drug Formulation and Delivery"**, invited talk in the conference *Challenges in Global Product Development: Developing Rugged and Robust Products, Processes and Specifications*, March 31 - April 2, 2009, Bar Ilan University, Tel Aviv, Israel.

57. Panayiotis P. Constantinides **"Nanoparticles : Definitions and Product Development Considerations"**, invited panel talk at the Roundtable *Nanoparticles-Are They Ever Going to Amount to Anything*, 2009 AAPS Annual Meeting, November 8-12, 2009, Los Angeles, CA.

58. Panayiotis P. Constantinides "**Why, When and What Lipid-Based Formulations and Dosage Forms to Consider with BCS II and IV Compounds",** invited talk at the 2010 June LOL Pharmaceutical Conference on *Science-Driven Drug Product Development Strategies to Achieve Proof of Concept*, June 7-11, 2010, Merrimac, WI.

59. Panayiotis P. Constantinides **"Pharmaceutical Nanoparticulate Systems : Case Studies and Product Development Considerations",** invited keynote talk at the 3rd Annual Nanotechnology Symposium, September 24-25, 2010, Sullivan University, Louisville, KY.

60. Panayiotis P. Constantinides **"Enhancing Solubility, Intestinal Permeability and Bioavailability with Lipids: Principles and Case Studies with BCS II, III and IV Molecules**", seminar, November 19, 2010, Cubist Pharmaceuticals, Lexington, MA.

61. Panayiotis P. Constantinides, panelist in the workshop on **"Working with Industry: Designing Your Academic Research for Successful Collaboration: Part I, Basic Sciences Research"**, January 13, 2011, Rush University Medical Center, Chicago, IL.

62. Panayiotis P. Constantinides, organizer and panelist, panel on **"Commercializing Nanobiotechnology: Research, Development, Legal and Investment Perspectives"**, iBIO 2011 IndEx, February 15-16, 2011, Chicago, IL.

63. Panayiotis P. Constantinides **"Improving Poor Biopharmaceutical Properties of Small Molecules and Macromolecules with Lipids: Rationale, Achievements and Challenges",** invited talk at the 2011 AAPS Drug Delivery Workshop on *Emerging Oral Delivery Strategies and Technologies to Enable Biopharmaceutical Performance of BCS II, III and IV Molecules*, April 14-15, 2011, Baltimore, MD.

64. Panayiotis P. Constantinides **"Product Development Considerations with Targeted Nanotheragnostics",** invited talk at the symposium on *Targeted Nanotheragnostics : Scientific Achievements and Commercialization Challenges*, 2011 AAPS National Biotechnology Conference, May 16-18, 2011, San Francisco, CA.

65. Panayiotis P. Constantinides **"Solubility and/or Permeability Enhancement Using Lipids: Case Studies"**, invited talk at the 2011 June LOL Pharmaceutical Conference on *Solubility and Bioavailability Enhancement: Product Development Strategies for Classic Challenges*, June 6-10, 2011, Merrimac, WI.

66. Panayiotis P. Constantinides "**Nanoparticle Strategies in Cancer Drug Delivery: Biopharmaceutical Perspectives"**, Plenary Lecture, 4th Annual Nanotechnology Symposium, September 23 - 24, 2011, Sullivan University, Louisville, KY.

67. Panayiotis P. Constantinides **"Pharmaceutical Nanoparticulate Systems: Principles, Case Studies and Product Development Considerations",** invited speaker by the AAPS Greater Maryland Discussion Group (GMDC), October 11, 2011, University of Maryland, School of Pharmacy, Baltimore, MD.

68. Panayiotis P. Constantinides, organizer, moderator and panelist on **"Solid Self-Emulsified Water-in-Oil Microemulsions, Reverse Micelles and Nanoparticles"**, Roundtable on *Transforming Oral Liquid Lipid Formulations of BCS II, III and IV Molecules to Solid Dosage Forms: Prospects and Challenges*, 2011 AAPS Annual Meeting, October 23-27, 2011, Washington, DC.

69. Panayiotis P. Constantinides **"Pharmaceutical Emulsions and Related Systems for Oral and Topical Administration: Principles, Characterization and Cases Studies"** seminar, December 2, 2011, Merck Consumer Care, Memphis, TN.

70. Panayiotis P. Constantinides **"Pharmaceutical Nanoparticulate Systems: Principles, Case Studies and Product Development Considerations"**, webinar, December 13, 2011 Johnson & Johnson Science Forum/Delivery Technology Update.

71. Panayiotis P. Constantinides **"Lipidic Nanoparticulates : Design and Development Considerations and Case Studies"**, invited talk at the 47th AAPS Arden House Conference *Nanoscience in Pharmaceuticals: Translating Fundamental Understanding to Practical Application in Drug and Device Development*, March 11-14, 2012, The Thayer Hotel, West Point, NY.

72. Panayiotis P. Constantinides, **"Enabling Oral Drug Delivery with Lipids : Trends and Perspectives"** invited talk at the 2012 AAPS Workshop *Lipid-Based Delivery for Improving Drug Absorption : Mechanistic Understanding and Practical Approaches,* April 23-24, 2012, Baltimore, MD.

73. Panayiotis P. Constantinides, **"Parenteral and Oral Lipid Nanodispersions for Small Molecules and Macromolecule Delivery: Biopharmaceutical Considerations and Case Studies",** invited talk at the symposium *Advances in Lipid-Based Nanoparticulate Systems for Drug and Vaccine Delivery*, CSPS Conference "Modern Therapeutics 2012: Advances in Physiology, Pharmacology & Pharmaceutical Sciences", June 12-15, 2012, Toronto, Canada.

74. Panayiotis P. Constantinides **"Nanoparticle Technologies in Drug Formulation and Delivery : Design and Development Considerations and Case Studies"**, invited distinguished speaker at the *Innovative Technologies in Healthcare* Global Seminar sponsored by Evonik, March 5-6, 2013, Mumbai, India.

75. Panayiotis P. Constantinides**, "Oral Lipid-Based Systems in Drug Development and Life Cycle Management"** invited quest lecture at VerGo Pharma and India Pharmaceutical Association, March 8, 2013, Verna, Goa, India.

76. Panayiotis P. Constantinides **"Advances in Nano Drugs for Cancer Chemotherapy : Biopharmaceutical Trends and Perspectives and Case Studies"**, keynote talk at the *3rd International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems (Pharmaceutica-2013)*, OMICS Group, April 8-10, 2013, Northbrook, IL.

77. Panayiotis P. Constantinides, **"Project, Product or Company : A CSO/CTO Perspective"**, invited talk and panelist at the *BIO 2013 Biotechnology Entrepreneurship Boot Camp* , Session 3, April 21-22, 2013, Chicago, IL.

78. Panayiotis P. Constantinides **"Entrepreneurship and Starting a New Business"** panelist in a roundtable on *Biotech Career Transition: Adaptability to Change and Strategies for Success*, (P.P. Constantinides and P. Ramsey, organizers), 2013 AAPS National Biotechnology Conference, May 20-22, 2013, San Diego, CA.

79. Panayiotis P. Constantinides **"The Scientific Basis for Fixed Dose Combination Products: Opportunities and Challenges"**, invited talk at the 2013 June LOL Conference on *Fixed Dose Combination Drug Development: Clinical, Formulation and Regulatory Challenges*, June 3-6, 2013, Madison, WI.

80. Panayiotis P. Constantinides **"Integrated Drug Discovery and Development Strategy and Models",** invited talk and co-chair, *Session II R&D in Drug Innovation*, Bio Eco 2013, June 25-26, 2013, Tianjin Institute of Pharmaceutical Research, Tianjin, China.

81. Panayiotis P. Constantinides **"Nanoparticles Technologies in Drug Delivery : Design and Development Considerations and Case Studies"** invited seminar at Nankai University, College of Life Sciences, June 26, 2013, Tianjin, China.

82. Panayiotis P. Constantinides **"Trends and Perspectives in Oral Lipid-Based Formulations and Dosage Forms for Immediate, Sustained/Controlled Drug Release",** invited seminar at CoSci Med-Tech Ltd, June 28, 2013, Beijing, China.

83. Panayiotis P. Constantinides **"Drug Emulsions/Microemulsions/Nanoemulsions : Quality Characteristics and Performance Assessment"**, invited talk in the mini-symposium *Overcoming Physical Stability Challenges with Multiphase Lipid Dispersed Systems and Impact on Product Quality and Performance*, 2013 AAPS Annual Meeting, November 10-14, 2013, San Antonio. TX.

84. Panayiotis P. Constantinides **"Enabling the Development and Life Cycle Management of Oral Poorly Soluble Drugs Using Lipid-Based Drug Delivery Systems",** invited keynote talk at the *4th International Conference and Exhibition on Pharmaceutics and Novel Drug Delivery Systems*, March 24-26, 2014 San Antonio, TX.

85. Panayiotis P. Constantinides **"Parenteral and Oral Nanoemulsions and Nanosuspensions: Processing, Characterization and In Vitro/In Vivo Performance Assessment",** invited talk and Chair, *Solubility and Bioavailability Enhancement Workshop : Accelerating Translation of Challenging Compounds from Discovery to Clinical Testing and the Market*, Ascendia Pharmaceuticals, LLC, April 28, 2014, North Brunswick, NJ.

86. Panayiotis P. Constantinides **"Solubility and Bioavailability Enhancement in Drug Development : Drivers, Technologies to Consider and Assessment Strategies",** invited keynote talk, Evonink's *5th Solubility and Bioavailability Enhancement Symposium Utilizing Pharmaceutical Melt Extrusion and Spray Drying Techniques*, May 13-14, 2014, South Plainfield, NJ.

87. Panayiotis P. Constantinides **"Advances in Parenteral Drug Nanodispersions : Processing, Characterization and Case Studies",** invited talk at the *University of Nebraska Medical Center , Biopharma R&D Symposium*, June 5-6, 2014, Omaha, NE.

88. Panayiotis P. Constantinides **"Overview of Particle Engineering Needs in Pharma"**, co-organizer, speaker and panelist, Preconference Workshop on ***"Particle Engineering Technology Selection and Partnership: A Roadmap to Commercialization"***, 2014 June LOL Conference, *"Particle Engineering in API and Drug Product Design",* June 9-12, 2014, Madison, WI.

89. Panayiotis P. Constantinides **"Advancing Oral Peptide Formulations to Clinic and Commercialization : Development Considerations"**, invited talk at the Workshop *"Oral Peptide/Protein Delivery"*, July 12-13, 2014, CRS Annual Meeting, Chicago, Illinois.

90. Panayiotis P. Constantinides **"Oral Emulsions, Microemulsions and Reverse Micelles for BCS II/IV and III Molecules",** invited talk at the AAPS *Workshop Improving Bioavailability by Lipid-Based Delivery Approaches*, November 1-2, 2014, San Diego, CA.

91. Panayiotis P. Constantinides **"Excipients as Inhibitors of the Absorption of Dietary Cholesterol : Principles and Case Studies with Nanostructured Aluminosilicates",** invited

talk at the symposium on *Excipients as Atypical Actives in Nutraceuticals and Pharmaceuticals : Applications and Development Considerations*, 2014 AAPS Annual Meeting, November 2-6, 2014, San Diego, CA.

92. Panayiotis P. Constantinides **"Oral Lipid-Based Formulations as Enabling Strategy in Drug Discovery and Life Cycle Management"**, invited speaker and member of the organizing committee, *1st International Congress of Controlled Release Society-Greek Local Chapter*, May 27-28, 2015, Athens, GREECE.

93. Panayiotis P. Constantinides **"Non-Traditional Uses of Pharmaceutical Excipients : Applications and Development Considerations",** invited speaker, 1st International Workshop of MD Pharmacon Pharmaceutical Services Ltd on *Advances in Scientific-Regulatory Issues in Drug Development and Authorization Processes*, May 29, Athens, Greece.

94. Panayiotis P. Constantinides **"Parenteral Drug Nanodispersions : Manufacturing, Characterization and *In Vitro/In Vivo* Performance Evaluation",** keynote talk at OMICS Parenterals-2015 Conference, August 17-19, 2015, Chicago, Illinois.

95. Panayiotis P. Constantinides **"Addressing Solubility and/or Permeability Challenges in Drug Development : Best Practices",** speaker and organizer, short course on *Assessing and Applying Enabling Delivery Technologies and Formulation Tools to Oral Small Molecule (BSC II/IV) and Peptide Therapeutics (BCS III)*, 2015 AAPS Annual Meeting, October 25-29, Orlando, FL.

96. Panayiotis P. Constantinides **"Starting a New Pharma/Biotech Company or Consulting Business: Why, What and How?"** professional development mini-session, 2015 AAPS Annual Meeting, October 25-29, Orlando, FL.

97. Panayiotis P. Constantinides **" Effective Use of Lipids in Drug Delivery : Key Considerations and Best Practices"** invited talk at the Workshop *Enabling the Development of Oral Therapeutics with Innovation in Lipid Formulation Technologies*, September 19-20, 2016, Plainsboro, NJ.

98. Panayiotis P. Constantinides **"Dispersed Systems for Oral Peptides Incorporating Permeation Enhancers and Peptide Stabilizers"** invited panelist in a dialogue and debate session *Getting Oral Peptides to the Market : To Conjugate and/or Disperse the Peptide?*, 2016 AAPS Annual Meeting, November 13-17, 2016, Denver, CO.

99. Panayiotis P. Constantinides **"Oral Lipid Formulation Case Studies: Linking Drug Product Quality to Performance",** invited talk at the 2017 June Land O'Lakes Pharmaceutical Conference, *Material Sciences Approaches to Improving Drug Delivery Performance: From Discovery Through Manufacturing*, June 5-8, 2017, Madison, WI.

100. Panayiotis P. Constantinides **"Evolution of Excipient Use in Drug Products: Traditional and Non-traditional Uses and Implications in Excipient Selection and Formulation Development",** invited talk AAPS Short Course *Pharmaceutical Excipients: Biopharmaceutical, Quality Control and Regulatory Considerations*, 2017 AAPS Annual Meeting, November 12-15, 2017, San Diego, CA.

101. Panayiotis P. Constantinides **"Academia-Industry Partnerships: Success Considerations and Lessons Learned"** invited talk in the symposium *Academia-Biotech/Pharma Industry*

Partnerships: Opportunities and Challenges, 2017 AAPS Annual Meeting, November 12-15, 2017, San Diego, CA.

102. Panayiotis P. Constantinides **"Development and Commercialization of Oral Peptides/Proteins: Trends and Perspectives"**, keynote talk at the *12th World Drug Delivery Summit*, September 24-26, 2018, Chicago, IL.

103. Panos P. Constantinides and Ronak Savla, co-presenters, **"Is an Oral Lipid-Based Formulation Best for Your Molecule? *Insights from exclusive survey on strategies for poorly soluble molecules",*** March 27, 2019 Catalent Webinar.

Issued US and EP Patents

1. Laman A. Al-Razzak, Panayiotis P. Constantinides, Dilip Kaul, John Lipari, Lisa McChesney-Harris and Bashar Y. Abdullah **"Hydrophilic Binary Systems for the Administration of Lipophilic Compounds"**, ***US Patent 6,008,192 , December 28, 1999***. This invention discloses binary pharmaceutical compositions comprising (i) a cyclosporine compound, (ii) a hydrophilic phase and (iii) a surfactant, provide bioavailability of the active ingredient which is equivalent to that provided by ternary compositions, but without the need for a lipophilic phase.

2. Karel Lambert, Panayiotis P. Constantinides and Steven C. Quay **"Emulsion Vehicle for Poorly Soluble Drugs", *US 6,458,373, October 1, 2002***. An emulsion of α-tocopherol, stabilized by biocompatible surfactants, as a vehicle or carrier for therapeutic drugs, which is substantially ethanol-free and which can be administered to animals or humans by various routes is disclosed. Also included in the emulsion is PEGylated vitamin E. PEGylated α-tocopherol includes polyethylene glycol subunits attached by a succinic acid diester at the ring hydroxyl of vitamin E and serves as a primary surfactant, stabilizer and a secondary solvent in emulsions of α-tocopherol.

3. Panayiotis P. Constantinides, Karel Lambert, Alexander Tustian and Andrew Nienstedt, **" Compositions of Tocol-Soluble Therapeutics"**, ***US 6,479,540, November 12, 2002***. Tocol-based compositions of charged amphiphilic and water soluble pharmaceutically active compounds or their charged precursors are prepared by forming a tocol-soluble ion pair with an oppositely charged ion-pair forming compound capable of forming a tocol-soluble ion-pair with the active compound. Also disclosed are novel compounds tocopherolsuccinate-aspartate and tocopherolsuccinate-glutamate, which are useful as ion-pair forming compounds.

4. Karel Lambert, Panayiotis P. Constantinides, Alex Tustian and Steven C. Quay, **"Emulsion Vehicle for Poorly Soluble Drugs", *US 6,660,286, December 9, 2003***. An emulsion incorporating one or more tocols, a co-solvent and, stabilized by biocompatible surfactants, as a vehicle or carrier for therapeutic drugs, which is substantially ethanol free and which can be administered to animals or humans by various routes is disclosed. Also included in the emulsion is PEGylated vitamin E, PEGylated α-tocopherol includes polyethylene glycol subunits attached by a succinic acid diester at the ring hydroxyl of vitamin E and serves as a primary surfactant, stabilizer and a secondary solvent in tocol emulsions.

5. Karel Lambert, Panayiotis P. Constantinides, Alex Tustian and Steven C. Quay, **"Emulsion Vehicle for Poorly Soluble Drugs",** US 6,667,048, December 12, 2003.

6. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same"**, US 8,241,664, August 14, 2012.

7. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same",** EP1871384, 1/2/2008.

8. Panayiotis P. Constantinides, Likan Liang and Eun-Hyun Jang, **"Stabilized Reverse Micelle Compositions and Uses Thereof",** EP 1460992, 3/11/2009.

9. Panayiotis P. Constantinides, Likan Liang and Eun-Hyun Jang, **"Stabilized Reverse Micelle Compositions and Uses Thereof",** US 8,535,650, September 17, 2013.

10. Arthur Michaelis and Panayiotis P. Constantinides, **"Pharmaceutical Compositions and Uses Thereof"**, EP2056835, 5/13/2009.

11. Jerald W. Darlington and Panayiotis P. Constantinides, **"Cholesterol-Interacting Layered Phyllosilicates and Methods of Reducing Hypercholesteremia in a Mammal"**, EP2167069, 10/26/2011. Layered phyllosilicates are useful for adsorbing and/or binding to cholesterol and, thereby, reducing blood cholesterol in a patient. Accordingly, provided herein isa method of reducing hypercholesteremia in a mammal comprising administering to said mammal a layered phyllosilicate material alone and in combination with other cholesterol-reducing agents in an amount effective to reduce hypercholesteremia in said mammal.

12. Jerald W. Darlington and Panayiotis P. Constantinides, **"Cholesterol-Interacting Layered Phyllosilicates and Methods of Reducing Hypercholesteremia in a Mammal"**, US 8,481,084, July 9, 2013.

13. Robert E. Dudley and Panayiotis P. Constantinides, **"Oral Testosterone Ester Formulations and Methods of Treating Testosterone Deficiency Comprising Same"**, US 8,492,369, July 23, 2013.

14. Robert E. Dudley and Panayiotis P. Constantinides, **"Oral Testosterone Ester Formulations and Methods of Treating Testosterone Deficiency Comprising Same"**, US 8,778,916, July 15, 2014.

15. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same"**, US 8,778,917, July 15, 2014.

16. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same"**, US 8,828,428, September 9, 2014.

17. Wael Salameh and Panayiotis P. Constantinides **"Use of Oral Pharmaceutical Products Combining Testosterone Esters with Hypolipidemic Agents**", US 10,245,273, April 2, 2019.

18. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same",** US 11,179, 402, November 23, 2021.

19. Robert E. Dudley and Panayiotis P. Constantinides, **"Oral Testosterone Ester Formulations and Methods of Treating Testosterone Deficiency Comprising Same"**, US 11, 179, 403, November 23, 2021.

20. Christopher Kevil, Anthony Giordano, Douglas R. Flanagan and Panayiotis P. Constantinides **"Pharmaceutical Formulations of Nitrite and Uses Thereof",** US 10,307,441, June 4, 2019.

21. Christopher Kevil, Anthony Giordano, Douglas R. Flanagan and Panayiotis P. Constantinides **"Pharmaceutical Formulations of Nitrite and Uses Thereof",** US 10,463,689, November 5, 2019.

WO Patents

A. The following patents disclose **"Microemulsion Compositions with Improved Drug Delivery/Absorption Characteristics"** Drug Delivery Department, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania.

1. Panayiotis P. Constantinides, **"W/O Microemulsions"**, **WO 93/02664, 18 February 1993,** describes water-in-oil microemulsion compositions where the lipophilic phase is comprising of medium-chain glycerides (mono-, di-, and triglycerides).

2. Panayiotis P. Constantinides, **"W/O Microemulsions", WO 93/02665, 18 February 1993,** describes water-in-oil microemulsion compositions where the lipophilic phase is comprising of long-chain glycerides and sorbitan esters.

3. Panayiotis P. Constantinides, **"Compositions", WO 94 / 08603, 28 April 1994,** describes water-in-oil microemulsions where the lipophilic phase is comprising interesterified medium- and long-chain glycerides or sorbitan esters.

4. Panayiotis P. Constantinides, **"Therapeutic Microemulsions", WO 94 / 08605, 28 April 1994,** describes microemulsion compositions where the lipophilic phase is comprising physical mixtures of medium- and long-chain glycerides or sorbitan esters.

5. Panayiotis P. Constantinides and Seang H. Yiv **,"Pharmaceutical Emulsion Compositions" WO 94 / 08610, 28 April 1994,** describes microemulsion compositions where the lipophilic phase is comprising in addition to glycerides (mono-, di-, triglycerides) or sorbitan esters, medium-chain fatty acids/salts.

6. Panayiotis P. Constantinides, **"Microemulsions Containing Pharmaceutical Compositions", WO 94/19000, 1 September, 1994,** describes pharmaceutical compositions in the form of microemulsions comprising an oil, a mixture of high and low HLB surfactants in which the high HLB surfactant comprises an aliphatic, aryl or aliphatic-aryl sulfate, sulfonate or sulfosuccinate or salt thereof, an aqueous phase and a biologically active agent**.**

7. Panayiotis P. Constantinides, **"Microemulsions Comprising Therapeutic Peptides", WO 94/19001, 1 September, 1994,** describes pharmaceutical compositions in the form of microemulsions comprising an oil, a mixture of high and low HLB surfactants in which the high HLB surfactant comprises a medium-chain alkyl/dialkyl sulfate, sulfonate or sulfosuccinate salt dissolved in a polyhydric alcohol, an aqueous phase and optionally further comprises a biologically active agent **.**

8. Panayiotis P. Constantinides, **"Pharmaceutical Compositions", WO94/19003, 1 September, 1994,** describes pharmaceutical compositions in the form of microemulsions comprising an oil, a mixture of high and low HLB surfactants in which the oil is a propylene glycol or polyol ester of medium-chain fatty acids, and high HLB surfactant comprises a medium-chain alkyl/dialkyl sulfate, sulfonate or sulfosuccinate salt dissolved in a polyhydric alcohol, an aqueous phase and optionally further comprises a biologically active agent**.**

9. Panayiotis P. Constantinides, **"Camptothecin Formulations", WO 95/08986, 6 April 1995.** This invention provides for the novel formulations of Camptothecin and its structurally related analogs in multilamellar or unilamellar vesicles. These novel formulations provide improved pharmacokinetics and pharmacodynamics for the compounds herein and thereby lowering the dose-dependent toxicity for use in anticancer treatments.

B. The following discloses **"Self-emulsifying lipid-based systems for oral administration of lipophilic drugs "**, Formulation Development Center, Pharmaceutical & Analytical R&D, Pharmaceutical Products Division, Abbott Laboratories, North Chicago, Illinois.

1. Laman A. Al-Razzak, Panayiotis P. Constantinides, Rong Gao, Dilip Kaul, John Lipari, Terrence Mazer and Lisa McChesney-Harris **"Lipophilic Binary Systems for the Administration of Lipophilic Compounds"**, **WO98/40051, 17 September 1998**. This invention discloses binary pharmaceutical formulations comprising (i) a cyclosporine compound, (ii) a lipophilic phase and (iii) a surfactant provide bioavailability of the active ingredient which is equivalent to that provided by ternary compositions, but without the need for a hydrophilic phase.

C. The following patents disclose were filed by SONUS Pharmaceuticals, Bothell, Washington.

1. Karel Lambert, Dean Kessler, Andrew Nienstedt, Greg Hartgraves and Panayiotis P. Constantinides **"Tocol-Based Compositions Containing Amiodarone", AU 9482601, 8 April 2002.**

2. Nagesh Palepu, Dean Kessler, Alexander K. Tustian, Steven C. Quay, Panayiotis P. Constantinides and Karel J. Lambert, **"Method for Treating Colorectal Carcinoma Using a Taxane/Tocopherol Formulation"**, **US2003087954, 8 May, 2003.**

D. The following patent applications filed with the US PTO by DOR BioPharma, disclose **Lipid Polymer Micelles (LPM™)** for the delivery of water-soluble drugs/peptides (1, 2) and **Lipid Polymer Emulsions (LPE™)** and **Polymer Lipid Particles (PLP™)** for the solubilization and delivery of water-insoluble drugs (3,4):

1. Eun-Hyun, Likan Liang and Panayiotis P. Constantinides, **"Reverse Micelle Compositions and Uses Thereof", WO 03047494, 12 June, 2003.**
2. Eun-Hyun, Likan Liang and Panayiotis P. Constantinides, **"Stabilized Reverse Micelle Compositions and Uses Thereof ", WO 03047493, 12 June, 2003.**
3. Panayiotis P. Constantinides, Likan Liang and Reena Patil, "**Lipid Particles and Suspensions and Uses Thereof ", WO 03057128, 17 July, 2003.**
4. Panayiotis P. Constantinides, Likan Liang, Reena Patil and Elijah Bolotin, **"Monoterpene Compositions and Uses Thereof ", WO 03057193, 17 July, 2003**

E. Co-inventor in the following published patent applications with client companies.

22. Robert E. Dudley and Panayiotis P. Constantinides, **"Pharmaceutical Delivery Systems for Hydrophobic Drugs and Compositions Comprising Same",** WO 113505A2, 26 October 2006. A drug delivery system for oral administration of hydrophobic drugs with enhanced and extended absorption and improved pharmacokinetics is provided. In one embodiment, formulation comprising testosterone and testosterone esters, e.g. testosterone palmitate, are disclosed. Methods of treating a hormone deficiency or effective male contraception with the inventive formulations are also provided.

23. Arthur Michaelis and Panayiotis P. Constantinides, **"Pharmaceutical Compositions and Uses Thereof"**, WO 117556A2, 18 October 2007. It features pharmaceutical compositions including Rifalazil, a surfactant, and a lipophilic antioxidant and methods of use thereof.

24. John Hughes, Jerald W. Darlington, Jr., and Panayiotis P. Constantinides, **"Virus-Interacting Layered Phyllosilicates and Methods of Inactivating Viruses on Animate and Inanimate Surfaces",** US 2007/ 0224293 A1, 27 September, 2007. Layered phyllosilicates are useful for adsorbing and/or binding to and, thereby, inactivating viruses. Accordingly, provided herein is a method of inhibiting transfer of a virus to a surface comprising contacting the surface with a composition comprising a layered phyllosilicate material in an amount sufficient for inhibiting the transfer of the virus thereto. Also provided are methods of inactivating a virus on a surface comprising contacting the surface with a composition comprising a layered phyllosilicate material in an amount sufficient to inactivate said virus.

25. John Hughes, Panayiotis P. Constantinides, and Jerald W. Darlington, Jr. **"Virus-Interacting Layered Phyllosilicates and Methods of Inactivating Viruses in the Gastrointestinal Tract",** US 2007/ 0231412 A1, 4 October, 2007. Layered phyllosilicates are useful for adsorbing and/or binding to and, thereby, inactivating viruses. Accordingly, provided herein is a method of inactivating a virus in the gastrointestinal tract of a mammalian subject comprising administering to said subject a composition comprising a layered phyllosilicate material in an amount effective for virus inactivation. Also provided are methods of treating a viral infection in the gastrointestinal tract of a mammalian subject. Methods of delivering a therapeutic agent to a mammalian subject and methods of inactivating a virus in waste expelled from a mammal are also provided.

26. Christopher Kevil, Anthony Giordano, Douglas R. Flanagan and Panayiotis P. Constantinides **"Pharmaceutical Formulations of Nitrite and Uses Thereof",** US2011/0086069 A1, April 14, 2011. The present invention relates to pharmaceutical compositions of nitrites such as inorganic nitrites, or any pharmaceutically acceptable salts, solvates, or prodrugs thereof, and the medical use of these compositions. The pharmaceutical compositions, which can be formulated for oral administration, can provide immediate release or extended release of the nitrite ion (NO_2^-). The pharmaceutical compositions of the invention are useful, for example, for the treatment of chronic ischemia.

27. Christopher Kevil, Anthony Giordano, Douglas R. Flanagan and Panayiotis P. Constantinides **"Pharmaceutical Formulations of Nitrite and Uses Thereof",** EP10824097, 31 July 2013.

28. Wael Salameh and Panayiotis P. Constantinides **"Use of Oral Pharmaceutical Products Combining Testosterone Esters with Hypolipidemic Agents**", WO 2015/100406.

29. Robert E. Dudley, Panayiotis P. Constantinides and James A. Longstreth **"Methods of Treating Testosterone Deficiency"**, US 2017/0246184, August 31, 2017.

EXHIBIT 2

Basic Principles of Pharmacokinetics

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Basic Principles of Pharmacokinetics

Leslie Z. Benet and Parnian Zia-Amirhosseini

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Basic Principles of Pharmacokinetics*

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ABSTRACT

Pharmacokinetics may be defined as what the body does to a drug. It deals with the absorption, distribution, and elimination of drugs but also has utility in evaluating the time course of environmental (exogenous) toxicologic agents as well as endogenous compounds. An understanding of 4 fundamental pharmacokinetic parameters will give the toxicologic pathologist a strong basis from which to appreciate how pharmacokinetics may be useful. These parameters are clearance, volume of distribution, half-life, and bioavailability.

Keywords. Clearance; volume of distribution; half-life; bioavailability; extraction ratio

INTRODUCTION

An understanding of the basic principles of pharmacokinetics is necessary to appreciate how this discipline may serve as a tool for the toxicologic pathologist in understanding models that can be used for predicting and assessing drug-related toxic responses. Pharmacokinetics may be defined as what the body does to a drug. It deals with the absorption, distribution, and elimination of drugs but also has utility in evaluating the time course of environmental (exogenous) toxicologic agents as well as endogenous compounds. A fundamental hypothesis of pharmacokinetics is that a relationship exists between a pharmacologic or toxic effect of a drug and the concentration of that drug in a readily accessible site of the body (e.g., blood). This hypothesis has been documented for many drugs (5, 6), although for some drugs no clear relationship has, as yet, been found between pharmacologic effect and plasma or blood concentrations. An understanding of 4 fundamental pharmacokinetic parameters will give the toxicologic pathologist a firm basis from which to appreciate how pharmacokinetics may be useful. These parameters are clearance, a measure of the body's ability to eliminate drug; volume of distribution, a measure of the apparent space in the body available to contain the drug; half-life, a measure of the time required for a substance to change from one concentration to another; and bioavailability, the fraction of drug absorbed as such as the systemic circulation. These 4 parameters will be discussed here in detail. A number of classic pharmacokinetic texts may be consulted for further elucidation of these and other more detailed principles (4, 5, 8, 12, 15).

CLEARANCE

Clearance is the measure of the ability of the body to eliminate a drug. Clearance is expressed as a volume per unit of time. Clearance is usually further defined as blood clearance (CL_b), plasma clearance (CL_p), or clearance based on the concentration of unbound or free drug (CL_u), depending on the concentration measured (C_b, C_p, or C_u).

Clearance by means of various organs of elimination is additive. Elimination of drug may occur as a result of processes that occur in the liver, kidney, and other organs. Division of the rate of elimination for each organ by a concentration of drug (e.g., systemic concentration) will yield the respective clearance by that organ. Added together, these separate clearances will equal total systemic clearance:

$$CL_{hepatic} + CL_{renal} + CL_{other} = CL_{systemic}. \quad (1)$$

Other routes of elimination could include that in saliva or sweat, partition into the gut, and metabolism at sites other than the liver (e.g., nitroglycerin, which is metabolized in all tissues of the body).

Figure 1 depicts how a drug is removed from the systemic circulation when it passes through an eliminating organ. The rate of presentation of a drug to a drug-eliminating organ is the product of organ blood flow (Q) and the concentration of drug in the arterial blood entering the organ (C_A). The rate of exit of a drug from the drug eliminating organ is the product of the organ blood flow (Q) and the concentration of the drug in the venous blood leaving the organ (C_V). By mass balance, the rate of elimi-

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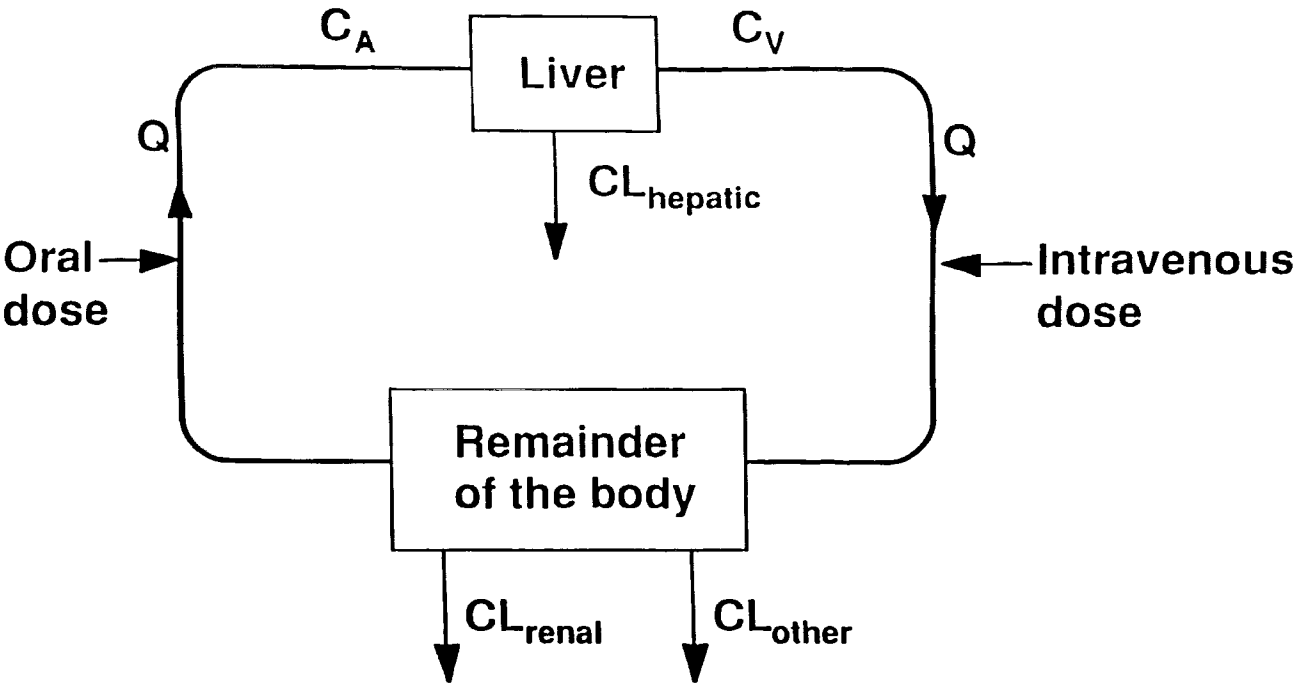

FIG. 1.—A schematic representation of the concentration–clearance relationship.

nation (or extraction) of a drug by a drug-eliminating organ is the difference between the rate of presentation and the rate of exit:

$$\text{rate of presentation} = Q \cdot C_A, \quad (2)$$

$$\text{rate of exit} = Q \cdot C_V, \quad (3)$$

$$\text{rate of elimination} = Q \cdot C_A - Q \cdot C_V = (C_A - C_V) \quad (4)$$

Extraction ratio (ER) of an organ can be defined as the ratio of the rate of elimination to the rate of presentation:

$$ER = \frac{Q \cdot (C_A - C_V)}{Q \cdot C_A} = \frac{(C_A - C_V)}{C_A} \quad (5)$$

The maximum possible extraction ratio is 1.0 when no drug emerges into the venous blood upon presentation to the eliminating organ (i.e., $C_V = 0$). The lowest possible extraction ratio is zero when all the drug passing through the potential drug-eliminating organ appears in the venous blood (i.e., $C_V = C_A$). Drugs with an extraction ratio more than 0.7 are by convention considered as high extraction ratio drugs, whereas those with an extraction ratio less than 0.3 are considered as low extraction ratio drugs.

The product of organ blood flow and extraction ratio of an organ represents a rate at which a certain volume of blood is completely cleared of a drug. This expression defines the organ clearance (CL_{organ}) of a drug.

$$CL_{organ} = Q_{organ} \cdot ER = Q_{organ} \cdot \frac{(C_A - C_V)}{C_A} \quad (6)$$

It is obvious from Equation 6 that an organ's clearance is limited by the blood flow to that organ (i.e., when ER = 1). Among the many organs that are capable of eliminating drugs, the liver has the highest metabolic capability. The liver may also clear drug by excretion in the bile. Kidney eliminates drugs primarily by excretion into the urine, but kidney metabolism may occur for some drugs.

Drug in blood is bound to blood cells and plasma proteins such as albumin and α_1-acid glycoprotein. Only unbound drug molecules can pass through hepatic membranes into the hepatocytes where they are metabolized by hepatic enzymes or transported into the bile. Thus, to be eliminated, the drug molecules must partition out of the red blood cells and dissociate from plasma proteins to become unbound or free drug molecules. The ratio of the unbound drug concentration (C_u) to total drug concentration (C) is defined as the fraction unbound (f_u):

$$f_u = \frac{C_u}{C} \quad (7)$$

Because an equilibrium exists between the unbound drug molecules in the blood cells and the plasma, the rate of elimination of unbound drugs is the same in the whole blood as in the plasma at steady state. Thus,

$$CL_p \cdot C_p = CL_b \cdot C_b = CL_u \cdot C_u, \quad (8)$$

where the subscripts p, b, and u refer to plasma, blood, and unbound, respectively.

Since the pioneering discussions of clearance in the early 1970s (11, 13), much has been made of the differences between high and low clearance (extraction ratio) drugs and the interpretation of the effects of pathological and physiologic changes on the kinetics of drug elimination processes. Utilizing the simplest model of organ elimination, designated the venous equilibration or well-stirred model, the blood clearance of an organ can be expressed according to the following relationship:

$$CL_{organ} = Q_{organ} \cdot \frac{(f_u)_b \cdot CL_{int}}{Q_{organ} + (f_u)_b \cdot CL_{int}} \quad (9)$$

Here, $(fu)_b$ represents fraction unbound in the blood and CL_{int} represents intrinsic clearance of the organ, that is, the ability of the organ to clear unbound drug when there are no limitations due to flow or binding considerations. Knowing that organ clearance is equal to the product of organ blood flow and extraction ratio of the organ (Equation 6), according to the well-stirred model, then

$$ER_{organ} = \frac{(f_u)_b \cdot CL_{int}}{Q_{organ} + (f_u)_b \cdot CL_{int}} \quad (10)$$

Examining Equations 9 and 10, one finds that for drugs with a low extraction ratio Q_{organ} is much greater than $(f_u)_b \cdot CL_{int}$; thus, clearance is approximated by $(f_u)_b \cdot CL_{int}$. However, in the case of a high extraction ratio drug (i.e., ER approaching 1.0), $(f_u)_b \cdot CL_{int}$ is much greater than Q_{organ}, and clearance approaches Q_{organ}. Therefore, the clearance of a high extraction ratio drug is perfusion rate–limited. Equations 11 and 12 describe these two cases:

If $Q_{organ} \gg (f_u)_b \cdot CL_{int}$, then

$$CL_{organ} \approx (f_u)_b \cdot CL_{int} \quad (ER < 0.3, \text{low ER}) \quad (11)$$

If $Q_{organ} \ll (f_u)_b \cdot CL_{int}$, then

$$CL_{organ} \approx Q_{organ} \quad (ER > 0.7, \text{high ER}) \quad (12)$$

Examples of low and high extraction ratio drugs are chlordiazepoxide and imipramine, respectively. The pharmacokinetic parameters for both drugs in humans are shown in Table I (6). Due to the low recovery in the urine (% excreted unchanged), one may assume that these drugs are mainly eliminated by the liver. Thus, hepatic extraction ratios for chlordiazepoxide and imipramine are 0.02 and 0.7, respectively. Note that the value of $(f_u)_b \cdot CL_{int}$ (35.8) for chlordiazepoxide is much lower than liver blood flow (1,500 ml/min) and conversely the value of $(f_u)_b \cdot CL_{int}$ for imipramine is more than twice the value of liver blood flow. Thus, elimination (clearance) of chlordiazepoxide is limited by fraction unbound and the intrinsic clearance of the liver, whereas that of imipramine is limited by liver blood flow.

TABLE I.—Pharmacokinetic parameters of chlordiazepoxide and imipramine in 70-kg humans.

Pharmacokinetic parameter	Chlordiazepoxide	Imipramine
CL (ml/min)	35	1,050
% Excreted unchanged	<1	<2
$ER_{hepatic}$	0.02	0.7
% Protein bound	96.5	94.8
F (%)	100	27
$t_{1/2}$ (hr)	10	18
V_{ss} (L)	21	1,600
CL_{int} (ml/min)	1,025	67,310
$(f_u)_b \cdot CL_{int}$	35.8	3,500

Elimination of both chlordiazepoxide and imipramine were studied in an *in vitro* rat microsomal system prepared from livers of rats that were injected with phenobarbital (an inducer of the P-450 enzyme family). In this *in vitro* system, the elimination of both drugs was higher in the phenobarbital-induced microsomes than in control microsomes. *In vivo* measured clearance of chlordiazepoxide in rats who had received phenobarbital was higher than the control rats (no phenobarbital administration). This is due to the fact that induction of enzymes by phenobarbital increases the hepatic CL_{int} and, because for this low extraction ratio drug $CL_{hepatic} \approx (f_u)_b \cdot CL_{int}$, a higher CL is measured in the presence of phenobarbital. In contrast, the *in vivo* measured clearance of imipramine in rats that received phenobarbital could not be differentiated from that measured in control rats. This is due to the fact that the value of $(f_u)_b \cdot CL_{int}$ for imipramine is already greater than liver blood flow. Thus, *in vivo*, liver blood flow is the limiting factor for the elimination of this drug and, because of this, enzyme induction will not substantially affect clearance of imipramine.

The ability of an organ to clear a drug is directly proportional to the activity of the metabolic enzymes in the organ. In fact, it is now well recognized that the product $(f_u)_b \cdot CL_{int}$ is the parameter best related to the Michaelis-Menten enzymatic saturability parameters of maximum velocity (V_{max}) and the Michaelis constant (K_m) as given in Equation 13, where C_{organ} is the total (bound + unbound) concentration of drug in the organ of elimination:

$$(f_u)_b \cdot (CL_{int}) = \frac{V_{max}}{K_m + C_{organ}} \quad (13)$$

Thus, only low extraction ratio drugs will exhibit saturable elimination kinetics following intravenous dosing. However, as shown subsequently in Equation 29, AUCs (area under the curves) following oral doses will be inversely related to CL_{int} for both high and low extraction ratio drugs.

Most drugs are administered on a multiple dosing

regimen, whereby after some time drug concentrations reach a steady-state level. At steady state, the rate of drug input to the body is equal to the rate of drug elimination from the body. The input rate is given by the dosing rate (dose/τ, where τ is the dosing interval) multiplied by the drug availability (F), whereas the rate of elimination is given by clearance multiplied by the systemic concentration (C). That is, at steady state,

$$\text{input rate} = \text{elimination rate}, \tag{14}$$

$$\frac{F \cdot (\text{dose})}{\tau} = (CL) \cdot (C) \tag{15}$$

When Equation 15 is integrated over all time from 0 to infinity, Equation 16 results:

$$F \cdot (\text{dose}) = (CL) \cdot (AUC), \tag{16}$$

where AUC is the area under the concentration–time curve and F is the fraction of dose available to the systemic circulation.

Thus, clearance may be calculated as the available dose divided by the AUC:

$$CL = \frac{F \cdot (\text{dose})}{AUC} \tag{17}$$

As described in the text following Equation 12, the maximum value for organ clearance is limited by the blood flow to the organ. The average blood flows to the kidneys and the liver are, respectively, approximately 72 and 90 L/hr.

Volume of Distribution

Volume of distribution (V) relates the amount of drug in the body to the concentration of drug in the blood or plasma, depending on the fluid in which concentration is measured. This relationship is defined by Equation 18:

$$V = \frac{\text{amount of drug in the body}}{C} \tag{18}$$

For an average 70-kg human, the plasma volume is 3 L, the blood volume is 5.5 L, the extracellular fluid outside the plasma is 12 L, and the total body water is approximately 42 L. However, many classical drugs exhibit volumes of distribution far in excess of these known fluid volumes. The volume of distribution for digoxin in a healthy volunteer is about 700 L, which is approximately 10 times greater than the total body volume of a 70-kg human. This serves to emphasize that the volume of distribution does not represent a real volume. Rather, it is an apparent volume that should be considered as the size of the pool of body fluids that would be required if the drug were equally distributed throughout all portions of the body. In fact, the relatively hydrophobic digoxin has a high apparent volume of distribution because it distributes predominantly into muscle and adipose tissue, leaving only a very small amount of drug in the plasma in which the concentration of drug is measured.

At equilibrium, the distribution of a drug within the body depends on binding to blood cells, plasma proteins, and tissue components. Only the unbound drug is capable of entering and leaving the plasma and tissue compartments. Thus, the apparent volume can be expressed as follows:

$$V = V_p + V_{TW}\frac{f_u}{f_{u,T}}, \tag{19}$$

where V_p is the volume of plasma, V_{TW} is the aqueous volume outside the plasma, f_u is the fraction unbound in plasma, and $f_{u,T}$ is the fraction unbound in tissue. Thus, a drug that has a high degree of binding to plasma proteins (i.e., low f_u) will generally exhibit a small volume of distribution. Unlike plasma protein binding, tissue binding of a drug cannot be measured directly. Generally, this parameter is assumed to be constant unless indicated otherwise.

Several volume terms are commonly used to describe drug distribution, and they have been derived in a number of ways. The volume of distribution defined in Equation 19, considers the body as a single homogeneous pool (or compartment) of body fluids. In this 1-compartment model, all drug administration occurs directly into the central compartment (the site of measurement of drug concentration, usually plasma), and distribution of drug is considered to be instantaneous throughout the volume. Clearance of drug from this compartment occurs in a first-order fashion, as defined in Equation 20; that is, the amount of drug eliminated per unit time depends on the amount (concentration) of drug in the body compartment. Figure 2A and Equation 20 describe the decline of plasma concentration with time for a drug introduced into this compartment:

$$C = \frac{\text{dose}}{V}\exp^{(-kt)}, \tag{20}$$

where k is the rate constant for elimination of the drug from the compartment. This rate constant is inversely related to the half-life of the drug ($k = 0.693/t_{1/2}$).

In this case (Fig. 2A), drug concentrations were measured in plasma 2 hr after the dose was administered. The semi-logarithmic plot of plasma concentration versus time appears to indicate that the drug is eliminated from a single compartment by a first-order process (Equation 20) with a half-life of 4 hr ($k = 0.693/t_{1/2} = 0.173\ hr^{-1}$). The volume of distribution may be determined from the value of

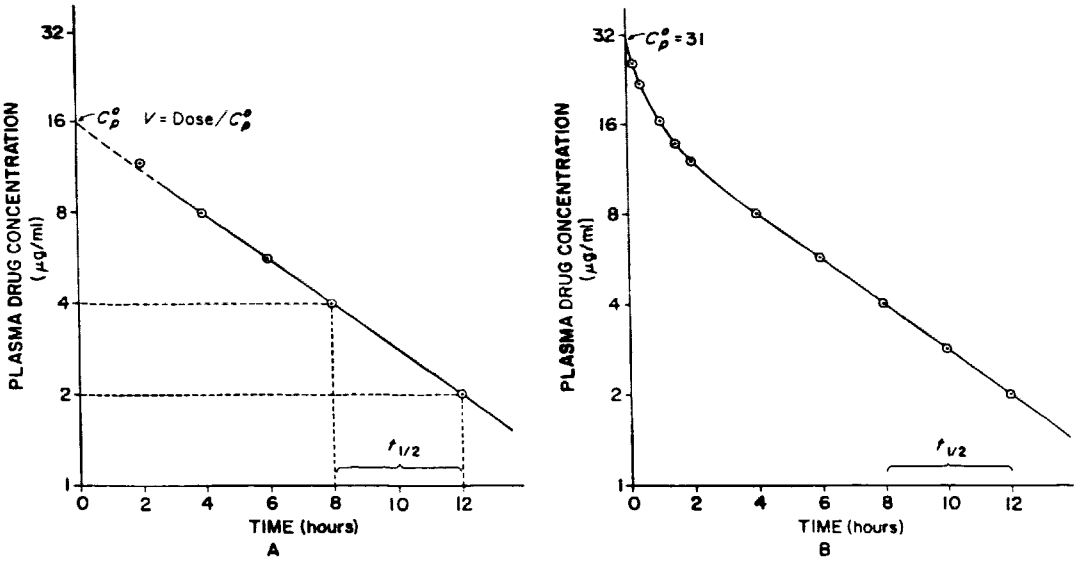

FIG. 2.—Plasma concentration–time curves following intravenous administration of a drug (500 mg) to a 70-kg human.

C_p obtained by extrapolation to t = 0 (C_p^0 = 16 μg/ml). In this example, the volume of distribution for the 1-compartment model is 31.3 L or 0.45 L/kg (V = dose/C_p^0). The clearance for this drug is 92 ml/min; for a 1-compartment model, CL = k·V.

For most drugs, however, the idealized 1-compartment model discussed earlier does not describe the entire time course of the systemic concentrations. That is, certain tissue reservoirs can be distinguished from the central compartment, and the drug concentration appears to decay in a manner that can be described by multiple exponential terms (Fig. 2B). Two different terms have been used to describe the volume of distribution for drugs that follow multiple exponential decay. The first, designated V_{area}, is calculated as the ratio of clearance to the rate constant describing the terminal decline of concentration during the elimination (final) phase of the logarithmic concentration versus time curve:

$$V_{area} = \frac{CL}{k} = \frac{(dose)}{k \cdot (AUC)} \qquad (21)$$

The calculation of this parameter is straightforward, and the volume term may be determined after administration of drug by intravenous or enteral routes (where the dose used must be corrected for bioavailability). However, another multicompartment volume of distribution may be more useful, especially when the effect of disease states on pharmacokinetics is to be determined. The volume of distribution at steady state (V_{ss}) represents the volume in which a drug would appear to be distributed during steady state if the drug existed throughout that volume at the same concentration as that in the measured fluid (plasma or blood). This volume can be determined by the use of areas, as described by Benet and Galeazzi (3):

$$V_{ss} = \frac{(dose)_{iv} \cdot (AUMC)}{(AUC)^2}, \qquad (22)$$

where AUMC is the area under the first moment of the curve that describes the time course of the plasma or blood concentration, that is, the area under the curve of the product of time t and plasma or blood concentration C over the time span 0 to infinity.

Although V_{area} is a convenient and easily calculated parameter, it varies when the rate constant for drug elimination changes, even when there has been no change in the distribution space. This is because the terminal rate of decline of the concentration of drug in blood or plasma depends not only on clearance but also on the rates of distribution of drug between the central and final volumes. V_{ss} does not suffer from this disadvantage (4).

In the case of the example given in Fig. 2, sampling before 2 hr indicated that the drug follows multiexponential kinetics. The terminal disposition half-life is 4 hr, clearance is 103 ml/min (calculated from a measurement of AUC and Equation 17), V_{area} is 28 L (Equation 21), and V_{ss} is 25.4 L (Equation 22). The initial, or "central," distribution volume for the drug (V = dose/C_p^0) is 16.1 L. This example indicates that multicompartment kinetics may be overlooked when sampling at early times is neglected. In this particular case, there is only a 10% error

in the estimate of clearance when the multicompartment characteristics are ignored. However, for many drugs multicompartment kinetics may be observed for significant periods of time, and failure to consider the distribution phase can lead to significant errors in estimates of clearance and in predictions of the appropriate dosage.

Volume of distribution is a useful parameter for determining loading doses. For drugs with long half-lives, the time to reach steady state (see the Half-Life section) is appreciable. In these instances, it may be desirable to administer a loading dose that promptly raises the concentration of drug in plasma to the projected steady-state value. The amount of drug required to achieve a given steady-state concentration in the plasma is the amount of drug that must be in the body when the desired steady state is reached. For intermittent dosage schemes, the amount is that at the average concentration. The volume of distribution is the proportionality factor that relates the total amount of drug in the body to the concentration in the plasma. When a loading dose is administered to achieve the desired steady-state concentration, then

$$\text{loading dose} = C_{p,ss} \cdot V_{ss} \qquad (23)$$

For most drugs, the loading dose can be given as a single dose by the chosen route of administration. However, for drugs that follow complicated multicompartment pharmacokinetics, such as a 2-compartment model (Fig. 2B), the distribution phase cannot be ignored in the calculation of the loading dose. If the rate of absorption is rapid relative to distribution (this is always true for intravenous bolus administration), the concentration of drug in plasma that results from an appropriate loading dose can initially be considerably higher than desired. Severe toxicity may occur, although transiently. This may be particularly important, for example, in the administration of antiarrhythmic drugs, where an almost immediate toxic response is obtained when plasma concentrations exceed a particular level. Thus, while the estimation of the amount of the loading dose may be quite correct, the rate of administration can be crucial in preventing excessive drug concentrations. Therefore, for drugs such as antiarrhythmics, even so-called "bolus" doses are administered by a slow "push" (i.e., no faster than 50 mg/min).

HALF-LIFE

The half-life ($t_{½}$) is the time it takes for the plasma concentration or the amount of drug in the body to be reduced by 50%. When drug is being administered as multiple doses or as a zero-order infusion, half-life also represents the time it takes for drug concentrations to reach one-half (or 50%) of the expected steady-state concentration. For the simplest case, the 1-compartment model (Fig. 2A), half-life may be determined readily and used to make decisions about drug dosage. However, as indicated in Fig. 2B, drug concentrations in plasma often follow a multiexponential pattern of decline; 2 or more half-life terms may thus be calculated.

Early studies of pharmacokinetic properties of drugs in disease states were compromised by their reliance on half-life as the sole measure of alterations of drug disposition. Only recently has it been appreciated that half-life is a derived parameter that changes as a function of both clearance and volume of distribution. A useful approximate relationship among the clinically relevant half-life, clearance, and volume of distribution is given by

$$t_{½} \cong (0.693) \cdot \frac{V}{CL} \qquad (24)$$

Equation 24 is exact for a drug following 1-compartment kinetics.

In the past, the half-life that was usually reported corresponded to the terminal log-linear phase of elimination. As greater analytical sensitivity has been achieved, the lower concentrations measured appeared to yield longer and longer terminal half-lives. For example, a terminal half-life of 53 hr is observed for gentamicin, and biliary cycling is probably responsible for a 120-hr terminal $t_{½}$ value reported for indomethacin. The relevance of a particular half-life may be defined in terms of the fraction of the clearance and volume of distribution that is related to each half-life and whether plasma concentrations or amounts of drug in the body are best related to measures of response (1).

Clearance is the measure of the body's ability to eliminate a drug. However, the organs of elimination can only clear drug from the blood or plasma with which they are in direct contact. As clearance decreases, due to a disease process, for example, half-life would be expected to increase. However, this reciprocal relationship is exact only when the disease does not change the volume of distribution. For example, the half-life of diazepam increases with increasing age; however, it is not clearance that changes as a function of age but the volume of distribution (10). Similarly, changes in protein binding of the drug may affect its clearance as well as its volume of distribution, leading to unpredictable changes in half-life as a function of disease. The half-life of tolbutamide, for example, decreases in patients with acute viral hepatitis, exactly the opposite from what one might expect. The disease appears to modify protein binding in both plasma and tissues, causing no change in volume of distribution

but an increase in total clearance because higher concentrations of free drug are present (14).

Although it can be a poor index of drug elimination, half-life does provide an important indication of the time required to reach steady state after a dosage regimen is initiated (i.e., 4 half-lives to reach approximately 94% of a new steady state), the time for a drug to be removed from the body, and a means to estimate the appropriate dosing interval.

If the dosing interval is long relative to the half-life, large fluctuations in drug concentration will occur. On the other hand, if the dosing interval is short relative to half-life, significant accumulation will occur. The half-life parameter also allows one to predict drug accumulation within the body and quantitates the approach to plateau that occurs with multiple dosing and constant rates of infusion. Conventionally, 3⅓ half-lives are used as the time required to achieve steady state under constant infusion. The concentration level achieved at this time is already 90% of the steady-state concentration (Table II), and, clinically, it is difficult to distinguish a 10% difference in concentrations.

TABLE II.—Percentages of steady-state concentration reached upon multiple dosing or during constant rates of infusion as a function of number of half-lives.

Number of half-lives	% Steady-state concentration
1.0	50.0
2.0	75.0
3.0	87.5
3.3	90.0
4.0	93.8
5.0	96.9

BIOAVAILABILITY

The bioavailability of a drug product via various routes of administration is defined as the fraction of unchanged drug that is absorbed intact and reaches the site of action, or the systemic circulation following administration by any route. For an intravenous dose of a drug, bioavailability is defined as unity. For drug administered by other routes of administration, bioavailability is often less than unity. Incomplete bioavailability may be due to a number of factors that can be subdivided into categories of dosage form effects, membrane effects, and site of administration effects. Obviously, the most available route of administration is direct input at the site of action for which the drug is developed. This may be difficult to achieve because the site of action is not known for some disease states, and in other cases the site of action is completely inaccessible even when drug is placed into the bloodstream. The most commonly used route is oral administration. Orally administered drugs may decompose in the fluids of the gastrointestinal lumen or be metabolized as they pass through the gastrointestinal membrane. Once a drug passes into the hepatic portal vein, it may be cleared by the liver before entering into the general circulation. The loss of drug as it passes through drug-eliminating organs for the first time is defined as the first-pass effect. For example, in Fig. 1, the availability of an oral dose of a drug eliminated by the liver will be less than an intravenous dose, due to the first-pass loss of drug through the liver following oral dosing. For high extraction ratio drugs, this first-pass loss, or decrease in oral bioavailability, will be markedly greater than for low extraction ratio drugs.

The fraction of an oral dose available to the systemic circulation considering both absorption and the first-pass effect can be found by comparing the ratio of AUCs following oral and intravenous dosing:

$$F = \frac{(AUC)_{oral}}{(AUC)_{iv}} \qquad (25)$$

Assuming the drug is completely absorbed intact through the gastrointestinal tract, and that the only extraction takes place at the liver, then the maximum bioavailability (F_{max}) is

$$F_{max} = 1 - ER_{hepatic} \qquad (26)$$

Combining Equations 6 and 26 results in the following relationship for maximum bioavailability:

$$F_{max} = 1 - \frac{CL_{hepatic}}{Q_{hepatic}} \qquad (27)$$

For high extraction ratio drugs, where $CL_{hepatic}$ approaches $Q_{hepatic}$, F_{max} will be small. For low extraction ratio drugs, $Q_{hepatic}$ is much greater than $CL_{hepatic}$; therefore, F_{max} will be close to 1.

The relationship between clearance and bioavailability for high and low extraction ratio drugs is summarized in Fig. 3. Equation 9 describes the simplest model for organ elimination and the approximate relationships or boundary conditions are given for high and low extraction substances (Equations 12 and 11, respectively). Substituting Equation 9 into Equation 27, yields Equation 28 as given in Fig. 3. The boundary conditions for F_{max} are also given. Much has been made of the comparison of clearance and bioavailability for high and low extraction compounds. However, in both therapeutics and toxicology, the primary concern will be with the actual exposure following an oral dose, because this is the measure of how much drug or toxic substance becomes available following ingestion by the most frequent route of administration. This measure of exposure (AUC) following oral dosing is given by

High Extraction **vs.** **Low Extraction**

$$CL_{organ} = Q_{organ} \bullet \frac{(f_u)_b \bullet CL_{int}}{Q_{organ} + (f_u)_b \bullet CL_{int}} \quad (9)$$

$CL_{organ} \approx Q_{organ}$ $CL_{organ} \approx (f_u)_b \bullet CL_{int}$

$$F_{max} = \frac{Q_{organ}}{Q_{organ} + (f_u)_b \bullet CL_{int}} \quad (28)$$

$F_{max} \approx \frac{Q_{organ}}{(f_u)_b \bullet CL_{int}}$ $F_{max} \approx 1$

$$(AUC_{oral,max})_b = \frac{Dose_{oral}}{(f_u)_b \bullet CL_{int}} \quad (29)$$

FIG. 3.—Critical equations utilizing the well-stirred model to define clearance, maximum oral bioavailability, and maximum area under the curve following an oral dose for high and low extraction ratio drugs.

Equation 29. Note that Equation 29 holds for both high and low extraction ratio compounds.

Equation 29 indicates that for drugs like chlordiazepoxide or imipramine, which are essentially completely absorbed and eliminated exclusively by hepatic metabolism, the area under the concentration versus time curve (AUC) is predicted by the oral dose divided by the fraction unbound $(f_u)_b$ and the intrinsic ability of the liver to eliminate the unbound drug (CL_{int}).

As discussed earlier, only the unbound drug can exert a pharmacologic effect. Thus, another important parameter to consider is the unbound area under the curve (AUC_u). If both sides of Equation 29 are multiplied by $(f_u)_b$, it can be seen in Equation 30 that the area under the curve unbound is a function only of the oral dose and the intrinsic ability of the liver to eliminate the drug:

$$(AUC_{u,oral,max})_b = \frac{dose_{oral}}{CL_{int}} \quad (30)$$

Because it is generally believed that pharmacodynamic response is related to unbound concentration, Equation 30 indicates that only the intrinsic ability of the liver to remove or clear unbound drug is the determining factor following an oral dose.

This is illustrated by data from hemodialysis patients for the nonsteroidal anti-inflammatory drug etodolac (7), which revealed a decrease in protein binding and total drug concentrations. No change in half-life was observed. Looking more carefully at data from a subgroup of 5 of these patients, there was no difference in unbound etodolac concentrations compared with normal subjects. Thus, although protein binding changes in hemodialysis patients, the unbound concentration does not change as predicted by Equation 30; therefore, altering etodolac dosage should not be necessary (2).

Recently, bioavailability and clearance data obtained from a cross-over study of cyclosporine kinetics before and after rifampin dosing revealed a new understanding of drug metabolism isozymes and the disposition of this compound (9). Healthy volunteers were given cyclosporine, intravenously and orally, before and after their cytochrome P-450 3A enzymes were induced by rifampin. As expected, the blood clearance of cyclosporine increased from 0.31 to 0.42 L/hr/kg due to the induction of the drug's metabolizing enzymes (i.e., an increase in

V_{max} in Equation 13). There was no change in volume of distribution, but there was a dramatic decrease in bioavailability from 27 to 10% in these individuals.

A decrease in bioavailability is to be expected, because cyclosporine undergoes some first-pass metabolism as it goes through the liver following oral dosing. But if one predicts on the basis of pharmacokinetics what the maximum bioavailability (as calculated by Equation 27 with an hepatic blood flow of 90 L/hr/70 kg) would be before and after rifampin dosing, the maximum bioavailability would decrease from 77 to 68%. Thus, there would be an expected cyclosporine bioavailability decrease of approximately 12% on the basis of the clearance changes resulting from inducing P-450 3A enzymes in the liver. In fact, there was a bioavailability decrease of 60%. Furthermore, bioavailability was significantly less than the predicted maximum bioavailability. While some of that lower bioavailability may be due to formulation effects, the discrepancy between the theoretical maximum bioavailability and the achievable bioavailability of cyclosporine remained a question.

On the basis of new findings during the last couple of years about the high prevalence of P-450 3A isozymes in the gut, significant metabolism of cyclosporine in the gut as well as in the liver was speculated. This hypothesis can consistently explain the significantly lower bioavailability than would be predicted even if all of the drug could be absorbed into the blood stream. This finding, particularly quantitation of the magnitude of gut metabolism (more than ⅔ of the total metabolism for an oral dose of cyclosporine occurs in the gut), would not have been realized had pharmacokinetics not been utilized in the analysis of the given data.

References

1. Benet LZ (1984). Pharmacokinetic parameters: Which are necessary to define a drug substance? *Eur. J. Resp. Dis.* 65(suppl. 134): 45–61.
2. Benet LZ (1994). Pharmacokinetics profile of etodolac in special populations. *Eur. J. Rheumatol. Inflam.* 14: 15–18.
3. Benet LZ and Galeazzi RL (1979). Noncompartmental determination of the steady-state volume of distribution. *J. Pharm. Sci.* 68: 1071–1074.
4. Benet LZ, Massoud N, and Gambertoglio JG (eds) (1984). *The Pharmacokinetic Basis for Drug Treatment.* Raven Press, New York.
5. Benet LZ, Mitchell JR, and Sheiner LB (1990). Pharmacokinetics: The dynamics of drug absorption, distribution, and elimination. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* AG Gilman, TW Rall, AS Nies, and P Taylor (eds). Pergamon Press, New York, pp. 3–32.
6. Benet LZ and Williams RL (1990). Design and optimization of dosage regimens; pharmacokinetic data. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* AG Gilman, TW Rall, AS Nies, and P Taylor (eds). Pergamon Press, New York, pp. 1650–1735.
7. Brater DC (1985). Renal safety profile of etodolac. In: *Etodolac: Clinical Perspectives in Antiarthritic Therapy,* TG Kantor (ed). Royal Society of Medicine Services Limited, London, pp. 37–43.
8. Gibaldi M and Perrier D (1982). *Pharmacokinetics,* 2nd ed. Marcel Dekker, New York.
9. Hebert MF, Roberts JP, Prueksaritanont T, and Benet LZ (1992). Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin. Pharmacol. Ther.* 52: 453–457.
10. Klotz U, Avant GR, Hoyumpa A, Schenker S, and Wilkinson GR (1975). The effects of age and liver disease on the disposition and elimination of diazepam in adult man. *J. Clin. Invest.* 55: 347–359.
11. Rowland M, Benet LZ, and Graham GG (1973). Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 1: 123–135.
12. Rowland M and Tozer TN (1989). *Clinical Pharmacokinetics,* 2nd ed. Lea & Febiger, Philadelphia.
13. Wilkinson G and Shand DG (1975). The physiological approach to hepatic blood clearance. *Clin. Pharmacol. Ther.* 18: 377–390.
14. Williams RL, Blaschke TF, Meffin PJ, Melmon KL, and Rowland M (1977). Influence of acute viral hepatitis on disposition and plasma binding of tolbutamide. *Clin. Pharmacol. Ther.* 21: 301–309.
15. Winter ME (1988). *Basic Clinical Pharmacokinetics,* 2nd ed. Applied Therapeutics, Spokane, Washington.

EXHIBIT 3

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Research Article

Case Studies for Practical Food Effect Assessments across BCS/BDDCS Class Compounds using *In Silico*, *In Vitro*, and Preclinical *In Vivo* Data

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Abstract. Practical food effect predictions and assessments were described using *in silico*, *in vitro*, and/or *in vivo* preclinical data to anticipate food effects and Biopharmaceutics Classification System (BCS)/Biopharmaceutics Drug Disposition Classification System (BDDCS) class across drug development stages depending on available data: (1) limited *in silico* and *in vitro* data in early discovery; (2) preclinical *in vivo* pharmacokinetic, absorption, and metabolism data at candidate selection; and (3) physiologically based absorption modeling using biorelevant solubility and precipitation data to quantitatively predict human food effects, oral absorption, and pharmacokinetic profiles for early clinical studies. Early food effect predictions used calculated or measured physicochemical properties to establish a preliminary BCS/BDDCS class. A rat-based preclinical BCS/BDDCS classification used rat *in vivo* fraction absorbed and metabolism data. Biorelevant solubility and precipitation kinetic data were generated *via* animal pharmacokinetic studies using advanced compartmental absorption and transit (ACAT) models or *in vitro* methods. Predicted human plasma concentration–time profiles and the magnitude of the food effects were compared with observed clinical data for assessment of simulation accuracy. Simulations and analyses successfully identified potential food effects across BCS/BDDCS classes 1–4 compounds with an average fold error less than 1.6 in most cases. ACAT physiological absorption models accurately predicted positive food effects in human for poorly soluble bases after oral dosage forms. Integration of solubility, precipitation time, and metabolism data allowed confident identification of a compound's BCS/BDDCS class, its likely food effects, along with prediction of human exposure profiles under fast and fed conditions.

KEY WORDS: absorption modeling; BCS/BDDCS; food effect prediction; human PBPK model; oral bioavailability.

INTRODUCTION

The effect of food on drug absorption can be mediated through various mechanisms, including enhancement in drug solubility, change of gastrointestinal (GI) pH and mobility, delayed stomach emptying, increased bile salt concentration, or direct interactions with the drug (1, 2). Food effects may significantly alter the systemic availability of orally dosed drugs which can impact pharmacological responses or safety margins (3–5). Fleisher, Wu, and Benet used the Biopharmaceutics Classification System (BCS; 6) to predict the direction and change in the extent of drug exposure affected by food (1, 7). The Biopharmaceutics Drug Disposition Classification System (BDDCS; 8, 9) which classifies compounds by solubility and metabolism has been applied for anticipating food effects (7). However, the BDDCS is apparently less frequently used by pharmaceutical scientists (8), possibly due to its perceived complexity. The BDDCS system assigns a drug classification using human *in vivo* drug metabolism data in lieu of human intestinal permeability to demonstrate that the extent of absorption is greater than 90% (7). Recently, Benet *et al.* utilized *in silico*/*in vitro* physicochemical parameters (e.g., LogP) to assign BDDCS class and probability of metabolism for new chemical entities (NCEs; 10). A BDDCS class could be used to identify likely food effects in early drug discovery stages. However, concentration–time profiles for clinical trials could not be predicted (e.g., C_{max} as peak plasma concentration or area under the curve (AUC) as area under the plasma concentration time curve) after various dosage forms. The BCS/BDDCS system may only provide a rough prediction for high-fat meal effects for drug products with limited formulation optimization. In reality, the effects of a meal on drug absorption and systemic exposure can be formulation dependent, and may be exacerbated with higher doses. The prediction of whether orally dosed drug product will show a food effect in human can be challenging, especially when an insoluble compound has undergone formulation optimization. In theory, a solubility-optimized

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formulation can convert a BCS class 2 drug to exhibit class 1 behavior when the dose number is reduced to less than 1 (6). For example, solid dispersions, nanocrystals, self-emulsifying drug delivery systems, and soluble cyclodextrin complexes have been successfully used to improve solubility for poorly water-soluble drugs (classes 2 or 4; 11). In such cases, predictions using the conventional BCS/BDDCS classes can fail or be inadequate. Traditionally, food effects have been assessed using dog *in vivo* studies with some success (12–15). However, the extent of food effect (changes on C_{max}, AUC) observed in dogs may not always translate directly to the human situation, and mechanistic understanding can be limited without the use of mechanistic models (16).

Recently, there has been a growing interest to evaluate the *in vivo* drug product performance with simulated pharmacokinetic (PK) profiles using physiologically based (PB) absorption models that are combined with a PK disposition model (17). Now, the integration of *in vitro*, *in silico,* and *in vivo* data is greatly facilitated due to advances of physiologically based pharmacokinetic (PBPK) modeling tools, such as GastroPlus (18, 19), Simcyp (20, 21), and STELLA (22–24). Integration of *in vitro* dissolution/solubility data generated in biorelevant media (25, 26) with *in silico* simulation tools (18, 19, 27, 28) may complement or even substitute animal models for quantitative assessment of the food effects trends. Although universally predictive food effect models remain elusive, even with advanced *in silico* advanced compartmental absorption transit (ACAT) models, appropriate model selection, and parameterization of biopharmaceutical properties can enable pharmaceutical scientists to predict food effect risks successfully. Here, several case examples related to practical food effect predictions and analyses across all BCS/BDDCS classes, using *in silico* predictions, *in vitro* biorelevant precipitation/solubility data, *in vitro* transporter kinetics, and *in vivo* animal models are described for all drug development stages.

MATERIALS AND METHODS

Computer Hardware and Software

GastroPlus (version 7.0, Simulations Plus, Inc, CA, USA) or Simcyp simulator (v11, Simcyp Limited, Sheffield, UK) were run on a Lenovo computer with Intel® Core™ i5 processor. These programs enable the prediction of rate and extent of oral drug absorption from the gastrointestinal tract using ACAT or the advanced drug absorption and metabolism (ADAM) model based on an absorption model originally established by Yu and Amidon (29). With the input of physicochemical properties (e.g., solubility, permeability, LogP, pKa, particle sizes), dissolution rates for solid formulations, and systemic PK parameters human concentration time profiles can be generated.

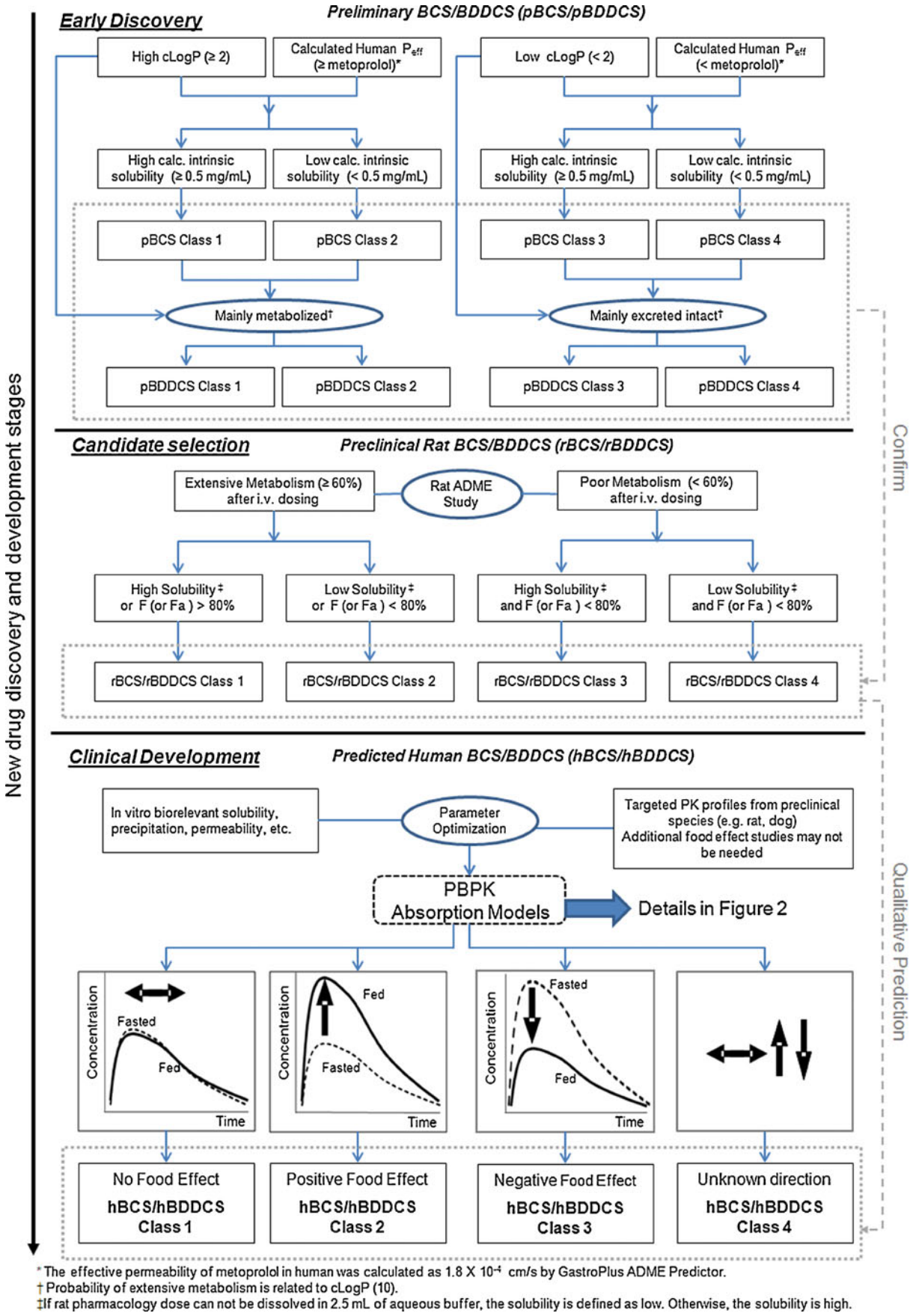
General Strategy for Human Food Effect Predictions

A flow chart for the qualitative and/or quantitative evaluations of food effects suitable for early discovery, drug candidate selection, and clinical development is shown in Fig. 1.

During early discovery stages, readily available calculated *in silico* or *in vitro* parameters may be used to define a preliminary BCS/BDDCS (pBCS/pBDDCS). The pBDDCS can be used to identify a food effect, as *in silico* predicted parameters, e.g., the calculated log of the octanol/water partition coefficient (clog P), correlated with the probability of extensive metabolism for orally dosed drug (10). The pBCS/pBDDCS class defined by *in silico*/*in vitro* parameters can be confirmed once metabolism and extent of absorption have been characterized in rats; the most commonly used preclinical species (Fig. 1). When *in vivo* radiolabeled mass balance and excretion studies have been conducted, a preclinical BCS/BDDCS classification based on rat data (rBCS/rBDDCS) can be established. The pBCS/pBDDCS and rBCS/rBDDCS, if consistent, will provide a rational estimate of human BCS/BDDCS class (hBCS/hBDDCS) to qualitatively predict likely human food effects. The practical approach to qualitatively predict or assess food effects from early discovery through candidate selection stages is described in Table I for seven Novartis compounds across BCS/BDDCS classes. The predictability of food effects using BCS/BDDCS relies on appropriate classification using multiple parameters (e.g., fraction absorbed, permeability, dose number, transporter effects, drug metabolism after intravenous dosing) as shown in Fig. 1 and Table I. As the conventional four class BCS/BDDCS cannot provide complete mechanistic information, food effects of some drug products may be not be predicted correctly, e.g., when drug delivery systems have been designed to improve oral absorption. To overcome these limitations and to predict human exposure profiles, physiologically based absorption models have been used to link drug products properties with *in vivo* performance (3).

Physiologically based models which integrate anatomical and physiological parameters of gastrointestinal tracts in preclinical species and humans, along with physicochemical drug product formulation properties, have been used to predict absorption and disposition (18–24, 30–32). These approaches have been applied during formulation development; yet their practical application in the prediction of human food effects has not been described extensively. Heimbach *et al.* reported the practical application of preclinical and clinical PK/PD modeling by integrating *in vitro* and preclinical *in vivo* data for the anticipation of human doses (30). Here, we used a similar strategy to predict potential food effect outcomes that can be conducted by both DMPK and formulation scientists. Briefly, the projection processes may include the following steps: (a) characterization of preclinical PK and related biopharmaceutical parameters under fasted and fed state; (b) correction interspecies differences; (c) scaling of preclinical *in vitro* and *in vivo* parameters to human situation; and (d) prediction of human PK profiles in presence or absence of meals.

Differences in physiological conditions between fasted and fed states can impact oral absorption and food effects (1). Therefore, physiological parameters, either obtained from the literature or from default ACAT values, must be carefully examined and selected. Solubility measured in biorelevant media can be more predictive for *in vivo* drug solubilization than solubility measured in aqueous buffers (33). *In vivo* dissolution in ACAT models can be calculated with modified



* The effective permeability of metoprolol in human was calculated as 1.8 X 10^{-4} cm/s by GastroPlus ADME Predictor.
† Probability of extensive metabolism is related to cLogP (10).
‡ If rat pharmacology dose can not be dissolved in 2.5 mL of aqueous buffer, the solubility is defined as low. Otherwise, the solubility is high.

Fig. 1. Flowchart for "practical" food effect predictions across BCS/BDDCS classes from early discovery through clinical development using *in silico*, *in vitro*, and preclinical *in vivo* data

Table I. *In silico*, *In vitro*, and *In vivo* Data to Predict the BCS/BDDCS Class of Seven Novartis Compounds

Parameter	NVS732	NVS406	NVS562	NVS701	NVS001	NVS169	NVS113
***In silico* preliminary BCS/BDDCS**							
Calculated log P, or $LogD_{pH=7.4}$	0.83	3.4	5.0	4.5	1.0 (log $D_{pH=7.4}$)	5.0	4.6
Calc. human perm., P_{eff} (10^{-4} cm/s)[a]	0.56 (low)	2.47 (high)	3.13 (high)	1.34 (low)	1.63 (low)	0.21 (low)	1.24 (low)
Calc. intrinsic solubility (mg/mL)[b]	14.1 (high)	0.0026 (low)	0.0036 (low)	0.0028 (low)	6.77 (high)	0.077 (low)	0.11 (low)
Probability of extensive metabolism	Low	High	High	High	Low	High	High
pBCS/pBDDCS class	3/3	2/2	2/2	4/2	3/3	4/2	4/2
Rat BDDCS (ADME study with radiolabeled compounds)							
Rat dose number[c]	2.8	1286	3214	857	0.007	6.59	51.4
F_a (%)[d]	87	45	>80	26–34	8.5	29	27
Elimination pathway in Rat (% of dose recovered after an i.v. administration)							
Urinary excretion of intact drug	26.9	0.1	0.5[e]	0.0	5.0	2.54	< 0.1
Biliary excretion + GI secretion	8.6	9.7	99.5[e]	15.3	77.7	15.4	35
Percent metabolism	64.3	90.2	N/A	84.7	17.3	81.3	65
rBCS/rBDDCS class	1	2	2	2	3	2	2
Human BCS/BDDCS							
Dose number for drug substances (DS)[f]	0.013	600	1500	400	0.003	3.07, 0.51 (ME)	24, 1.7 (SF)
F or F_a (%), fasted condition	F: 85	F: 20 (susp.)	F_a: 50–97 (G + pred.)	F_a<30 (G + pred.)	F: ~3.0	F_a >90 (ME) Simcyp pred.	F_a>92 (SF) G + pred.
Transporter effects	Weak P-gp substrate	P-gp substrate	Minimal P-gp effects	Weak P-gp substrate	Substrate for uptake transporters	P-gp substrate	Weak P-gp substrate
Pred. hBCS/BDDCS Class[g]	1	2	2	2	3	2	2
Pred. human food effects	No	Positive	Positive	Positive	Negative	No	No
Obs. human food effects[h]	No	Positive	Positive	Positive	Negative	No	Negative
Obs. hBCS/hBDDCS Class[g]	1	2	2	2	3	2[i]	4[j]

[a] Human effective permeability was calculated by GastroPlus ADMET predictor. Metoprolol was used as a high permeability marker, thus calculated P_{eff}>1.8×10^{-4} cm/s indicate high permeability
[b] Lowest water solubility (milligrams per milliliter) over the pH range 1–8 calculated by GastroPlus ADMET predictors. A value>0.5 mg/mL was defined as high solubility
[c] Dose number (rat)=human equivalent dose (milligrams per kilogram)/water solubility of drug substance (milligrams per milliliter)/maximum dose volume (10 mL/kg). The equivalent dose in rats is approximately 6 fold of the highest human dose tested in clinical food effects study
[d] Fraction absorbed calculated based on the following equation (41). If non-radiolabeled compounds are used, F_a can be estimated by equation of "$F_a = F/(1 - CL_b/Q_H)$ ", where Q_H (hepatic blood flow)=3.3 L/h/kg and CL_b is the blood clearance
[e] Compound related radioactivity recovered
[f] Dose number (human)=dose (milligrams)/water solubility of drug substance or formulation (milligrams per milliliter)/maximum dose volume (250 mL). Water solubility of each compound is listed in Table II
[g] The hBCS/BDDCS class is predicted based on estimated or observed human bioavailability for clinical service dosage form. The category of human BDDCS class for a compound can be formulation and dose dependent. The hBCS/BDDCS of a drug product is finalized or confirmed by the direction of observed human food effects
[h] The observed magnitudes for clinical food effects for each compound were listed in Table III
[i] Lack of food effect demonstrates potential class 1 behavior, due to low dose and optimized formulation
[j] Negative food effect may be the result of optimized solubility in fasted state
DS drug substance, *ME* microemulsion, *SF* solid formulation

Noyes–Whitney equations or be described by *in vitro* dissolution data (19). The latter approach predicted the dissolution process for formulations containing various excipients and complex matrices when *in vitro* and *in vivo* dissolution rate could be correlated (25, 26). Precipitation in the intestinal lumen can occur for poorly water soluble basic drugs at low gastric pH in the stomach (1, 22). With a meal, *in vivo* precipitation can be delayed due to a higher degree of supersaturation as the fed state pH is slightly lower and bile salt concentration is elevated (22). In the ACAT model, the precipitation process can be characterized by the precipitation time, which represents the time for drug particles to precipitate from solution when the local concentration exceeds the drug solubility. Direct measurements of *in vivo* precipitation time are impractical and difficult. Here, we describe how precipitation time can be estimated from physiologically based absorption models for BCS 2 drugs. An *in vitro* model for predicting the precipitation process of poorly soluble weak bases in the fasted and fed intestinal fluid has been reported, and a correlation between *in vitro* precipitation and *in vivo* absorption has been observed (34), suggesting that precipitation time could be estimated using *in vitro* methods. Alternatively, since dog studies are often used to predict human food effects (15), a method which estimates human precipitation time under fasted and fed condition by fitting PK data from dog food effect studies is proposed (Fig. 2).

For some drugs, absorptive transporters are involved in oral absorption. For a BCS class 3 drug which is a substrate for intestinal absorptive transporters, a "reverse pharmacokinetic" approach was used retrospectively to understand the underlying mechanism because the scaling factors of enzyme expression level from *in vitro* cell lines to the *in vivo* values in intestines remain unknown. *In vitro* Michaels–Menten kinetic data obtained from transfected expressed cells together with appropriated scaling factors (SF) that were optimized using existing clinical data under the fasted state are included in the PBPK model. When food inhibits absorptive transporters, SF can be optimized by fitting the observed fed PK profiles. This was done *via* increasing SF for the Michaels constant (K_m) or decreasing SF for maximal velocity of drug uptake (J_{max}). For BCS class 4 compounds, it is challenging to anticipate the direction of food effects, as food effects can be formulation and dose dependent as both solubility and permeability can be the rate limiting steps for absorption. For BCS 4 class drugs, our PBPK models obtained key absorption parameters (e.g., solubility and permeability) by fitting observed PK profiles from preclinical animal studies in which clinically relevant formulations had been administered prior to clinical trials. The optimized parameters were then used for prospective prediction of human food effects in human PBPK models. A general flow chart for quantitative food effect prediction using preclinical *in vitro* and *in vivo* data by physiologically based absorption modeling is shown in Fig. 2.

PBPK Modeling and Human Pharmacokinetics Predictions

Simulated human PK profiles were compared with observed data for each dose cohort from fasted and fed studies (Table III). Noncompartmental analysis was used to calculate PK parameters for predicted and observed human PK profiles using the WinNonlin Phoenix v6.1 (Pharsight, Sunnyvale, CA, USA). Area under the plasma concentration time curve (AUC) was calculated using trapezoidal calculation method, and peak plasma concentration (C_{max}) was directly determined from the observed and predicted plasma concentration time curves. The magnitude of food effect was measured in terms of the fold changes of AUC and C_{max} under fed state *versus* fasted state. The accuracy of prediction for food effect magnitude was evaluated by the fold error

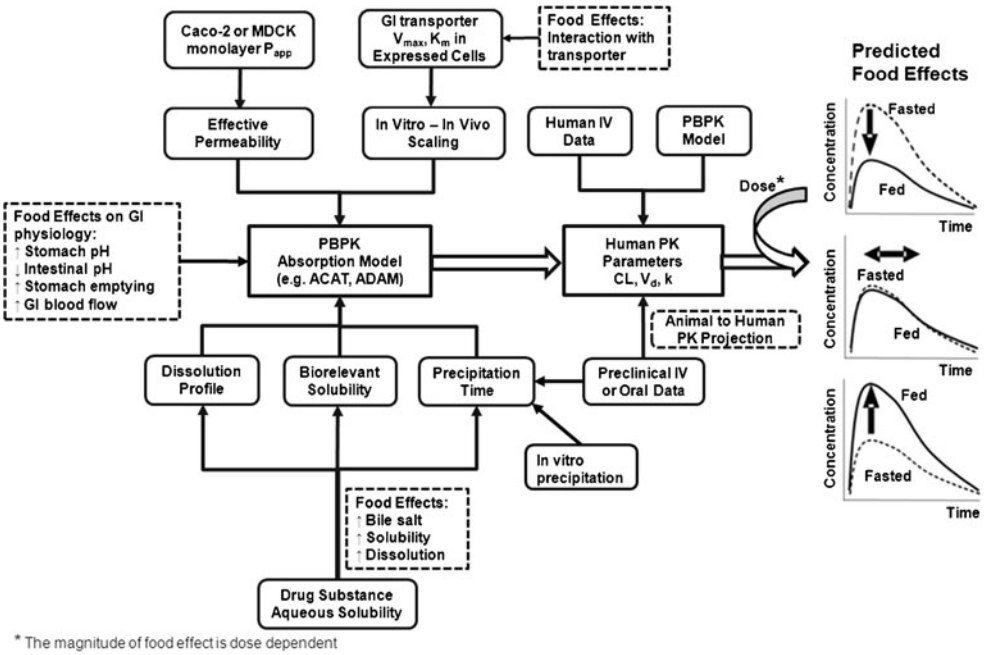


Fig. 2. General flowchart for quantitative food effect prediction using physiologically based absorption modeling

which has been published previously (Eq. 1; 33, 35–37). A prediction fold error that falls below 2 was defined as an accurate prediction.

$$FE = 10^{\left|\log\left(\frac{Predicted}{Observed}\right)\right|} \quad (1)$$

Models and Modeling Parameters Used

A PBPK model was established for each compound to simulate PK profile under fasted and fed condition, using available *in vitro* and *in vivo* parameters summarized in Table II for seven compounds. Table III lists the modeling approaches as "prospective prediction" or/and "retrospective analysis". The GastroPlus default human or dog physiology (Opt-Log D model) or Simcyp default ADAM model in v11 were used, except for lower gastric pH values (pH=2, default pH=3–5) in pentagastrin-treated dogs. The compound's PBPK disposition parameters were calculated directly from available intravenous PK profiles obtained from clinical studies or were projected from preclinical PK parameters and profiles (see Electronic Supplementary Material). For selected case studies, parameter sensitivity analyses were performed in two- or three-dimension response plots to explore the interaction between parameters and their influence on extent of absorption (F_a) under fasted and/or fed conditions.

Case Study 1: Food Effect Predictions for a BCS/BDDCS Class 1 Drug

NVS732 is a weak base with high solubility and moderate permeability (Tables II and III). The oral bioavailability (F) was moderate-to-high after dosing solutions with 94%, 64%, and 96%, in mice, rats, and dogs, respectively. Clinical food effect studies were conducted using a two-period, open label, single dose, and cross-over design in healthy volunteers. Subjects were randomized to receive single oral doses of 100 mg (fasted and fed). In this model, the default mean precipitation time of 900 s was used. The solubility of NVS732 was also measured in biorelevant media, i.e. simulated gastric fluid (SGF, pH1.6 with 0.08 mM sodium taurocholate), FaSSIF (pH6.5 with 3 mM sodium taurocholate), and FeSSIF (pH5 with 10 mM sodium taurocholate), and the measured values were incorporated in the ACAT model.

Case Study 2: Food Effect Prediction using Biorelevant Solubility Data

NVS406 is a weak base with low, pH-dependent solubility and high permeability. In human, PK studies after IV dosing were conducted and CL and V_{ss} were determined as 0.044 L/h/kg and 2.0 L/kg. NVS406 was mainly eliminated by hepatic metabolism and renal excretion was a minor elimination pathway (<10% of dose), regardless of the dosing route. A single dose of 150 or 450 mg was given as a coarse suspension formulation in fasted and fed condition in a clinical study. The default fasted or fed human ACAT physiology model was selected for the predictions. Solubility in different biorelevant media (SGF, FaSSIF, and FeSSIF) were measured and incorporated in ACAT model for the prediction of *in vivo* solubility in each gastrointestinal region.

Case Study 3: Food Effect Prediction using Two-Step Dissolution and Precipitation Data

NVS562 is a poorly soluble, lipophilic weak base (pKa= 5.0) with high calculated human effective permeability and high *in vivo* absorption (80%) in the rat (Table I). Formulations used in the clinical trials included a self-emulsifying solution (SEDDS) of the free base and a tablet of a stable salt. Solubility values for tablet or SEDDS formulations were measured in different biorelevant media (Table II). NVS562 dissolution and supersaturation behaviors were evaluated using a two-step dissolution method for each formulation. Initially, the drug release profile was investigated in 500 ml of simulated gastric fluid (pH1.6) using the U.S. Pharmacopeia (USP) paddle method at a rotating speed of 50 rpm at 37°C. After 60 min, the prewarmed (37°C) FaSSIF or FeSSIF medium (2×, 500 mL) was added and drug started to precipitate. A 5 mL sample of the solution was taken out periodically from 0 to 180 min, and the same amount of the medium at the same temperature was replaced. To quantitatively compare the precipitation kinetics of both formulations, the area under the curve ($AUC_{P,\ in\ vitro}$) and the area under the first moment-*versus*-time curve ($AUMC_{P,\ in\ vitro}$) of the remaining soluble compound (%) *versus* time in precipitation profile were calculated from 60 min to 180 min of the incubation time. A higher AUC for a precipitation profile represented less precipitation after medium changes. The *in vitro* mean precipitation time (MPT) was defined as the average time for the drug to solubilize or supersaturate in the solution, and was calculated by the Eq. 2.

$$MPT = \frac{AUMC_{P,in\ vitro}}{AUC_{P,in\ vitro}} \quad (2)$$

NVS562 human food effect was studied after a 50 mg single dose given as either SEDDS capsule or tablet under fasted and fed conditions. The calculated values of *in vitro* MPT were used as initial estimates of the *in vivo* precipitation time in the GastroPlus ACAT model.

Case Study 4: Food Effect Predictions Using the Dog Model for Weak Bases

NVS701 is a weak base with moderate to high permeability based on Caco-2 permeability and calculated human effective permeability (P_{eff}) as listed in Tables I and II. NVS701 has low and pH-dependent solubility (Tables I and II). A significant positive food effect was observed with a capsule formulation (capsule F1) of 200 mg dose as C_{max} and AUC increased by about twofold when administered 30 min after a high fat meal. As a positive food effect was undesirable, formulation approaches were proposed to slow drug precipitation in the intestine to maximize the bioavailability under the fasted state. A microemulsion formulation (Capsule F2) was developed with the expectation to provide higher solubility and to prevent precipitation. Both F1 and F2 capsules (50 mg) were tested in dogs under fasted and high fat meal conditions. GastroPlus ACAT models were

Table II. PBPK Input Modeling Parameters for Clinical Food Effect Prediction of Seven Novartis Compounds

PBPK Model Parameter	NVS732	NVS406	NVS562	NVS701	NVS001	NVS169	NVS113
Biopharmaceutical Properties							
Acid/Base	Base	Base	Base	Base	Acid	Ampholyte	Ampholyte
MW (g/mol)	~300	>350	>500	>500	>600	>600	>600
Measured Log D at pH 7.4	0.83	1.3	5.03	4.7	1.01	5.0	2.81
pKa[a]	7.4	3.7	5.0	3.5, 4.2, 6.2	8.4	5.8 (acid), 8.36 (base)	4.8 (acid), 4.2 (base)
Formulations	Tablet	Suspension	(1) SEDDS, (2) Tablet	(1) Capsule F1,(2) Capsule F2	Tablet	(1) DS, (2) ME	(1) DS, (2) SF
Obs. water solubility (mg/mL)	>30	0.003	0.0004	0.002	350	(1) 0.13, (2) 0.78 (fitted)[f]	(1) 0.005, (2) 0.07
SGF solubility at pH1.2 (mg/mL)	30	0.03	(1)2.48, (2) 2.3	2	350	(1) 0.65	(1) 0.0010, (2) 0.011
FaSSIF solubility at pH6.0	6.38	0.004	(1) 0.10, (2) 0.026	(1) 0.0083, (2) 0.05	N/A	(1) 0.23	(1) 0.0085, (2) 0.5
FeSSIF solubility at pH5.4	6.87	0.10	(1) 0.10, (2) 0.047	(1) 0.022, (2) 0.09	N/A	(1) 0.4	(1) 0.045, (2) 1.0
Caco-2 permeability (10^{-6} cm/s, A to B)	1.5	17	2.0	5.3	0.015	0.5; 3.7 (passive)[g]	2.4
Effective Human permeability[b](10^{-4} cm/s)	0.81	3.0	0.95	1.71, 6.15 (dog)	0.067	0.45, 1.78 (fitted)	1.0, 3.70 (dog)
Particle radius (μm)[c]	25	25	25	19	25	Set as 1	Set as 1
Diffusion Coefficients (cm^2/s)	0.771	0.75	0.697	0.65	0.49	0.402	0.506
Particle density (g/mL)	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Precipitation time (s)—fasted	900	900	(1) 6,000, (2) 6,000	(1) 1,800, (2) 2,500	900	900	900
Precipitation time (s)—fed	900	900	(1) 3,500, (2) 6,000	(1) 6,000, (2) 8,000	900	900	900
Pharmacokinetic properties[d]							
Human	2-comp. fit-obs.	3-comp. fit-obs.	2-comp. fit-pred.	2-comp. fit-pred.	3-comp. fit-obs.	2 -comp. fit-pred.	2 -comp. fit-pred.
Preclinical:	N/A	N/A	N/A	3-comp. fit (dog) obs.	N/A	2-comp. fit (rat) obs.	2-comp. fit (dog) obs.
Hepatic FPE[e] (%)	10	3.5	1.5	10, 30 (dog)	10	60, 95 (dog)	2, 2 (dog)
Fraction unbound in plasma (f_{up})	0.9	0.04	0.03	1.6, 0.9 (dog)	0.5	0.136	0.006
Blood to plasma partitioning ratio	0.97	0.73	0.58	0.68, 0.8 (dog)	0.83	0.62	0.57
Clearance (L/h/kg)	0.57	0.044	0.015	0.11, 0.54 (dog)	0.128	0.38	0.018
Volume of distribution (L/kg)	1	2	3.39	2.1, 2.5 (dog)	3.8	1.3	0.40

[a] Experimental pKa values were used for NVS732, NVS406, NVS562, NVS701, NVS001 and *in silico* calculated pKa values were used for NVS169 and NVS113

[b] The values of effective human permeability were calculated from measured Caco-2 apparent permeability using the GastroPlus converter. A value >1.8×10^{-4} cm/s (metoprolol) indicates high human permeability. Mannitol, a low permeability marker has a value of 0.3×10^{-4} cm/s

[c] The default value of 25 μm in GastroPlus built-in model was used as the particle radius if such information is not available. For NVS169 and NVS113, the particle radius was both set as 1 μm since oral solution or fine dispersion formulation were used respectively

[d] All pharmacokinetic parameter listed are values for human unless annotated. For clearance and volume values listed are either predicted (pred.) *via* allometry or are observed (obs.) clinical data

[e] First-pass extraction ($FPE=CL_b/Q_H$) where Q_H is the hepatic blood flow and CL_b is the blood clearance

[f] Bile salt dissolution model is not used in this model. The solubility is enhanced by the formulation and is assumed to be constant across the physiological pH range (1–8)

[g] The apparent Caco-2 permeability was measured in the presence of a potent P-gp inhibitor and the efflux ratio (B->A/A->B) was reduced from 50- to 1.5-fold

Table III. Summary of Observed and Predicted Food Effects (Represented as Fold Difference) for Seven Novartis Compounds with Respect to Area Under the Plasma Concentration–Time Curve (AUC_{0-last}) and Peak Plasma Concentration (C_{max})

				Human C_{max} ratio (fed/fasted)			Human AUC_{0-last} ratio (fed/fasted)			
Compound	Modeling approach[a]	Dose (mg)	Formulations	Predicted	Observed	Prediction fold error	Predicted	Observed	Prediction fold error	Dog observed AUC (C_{max}) Ratio (fed/fasted)
NVS732	Prospective	100	Tablet	0.84	0.90	1.07	0.99	0.88	1.12	
NVS406	Prospective	150	Suspension	4.84	6.11	1.26	3.98	4.35	1.09	Suspension: 5.57 (4.62)[d]; liquid form: 1.30 (0.99)[d]
NVS406	Prospective	450	Suspension	7.54	6.12	1.23	6.86	4.34	1.58	
NVS562	Pro./Retro.	50	SEDDS	0.82[b], 0.88[c]	0.85	1.04[b], 1.04[c]	0.97[b], 0.94[c]	1.01	1.04[b], 1.08[c]	
NVS562	Pro./Retro.	50	Tablet, salt	1.48[b], 1.77[c]	1.60	1.08[b], 1.11[c]	1.57[b], 1.70[c]	1.59	1.01[b], 1.07[c]	
NVS701	Prospective	200	Capsule F1	1.57	1.71	1.09	1.71	1.55	1.10	2.04 (1.73)[d]
NVS701	Prospective	200	Capsule F2	1.37	1.37	1.00	1.42	1.35	1.05	1.53 (1.39)[d]
NVS001	Retrospective	300	Tablet	0.28	0.28	1.00	0.27	0.31	1.15	0.14 (0.08)[e]
NVS169	Prospective	100	ME	0.96	0.52	1.84	1.14	1.01	1.13	
NVS113	Prospective	30	SF	0.93	0.60	1.53	0.97	0.71	1.37	1.09 (1.03)[d]

[a] Prospective prediction and/or retrospective analysis are done to analyze the human food effects. In this study, if the parameters used in the models are not optimized using observed human PK data in the food effect study, the modeling approach is considered as prospective prediction. Retrospective analysis can be used to refine the model if parameters in the model are unknown or can not be well characterized from experiments

[b] Predicted with the mean precipitation time fitted against the *in vivo* plasma concentration–time curve

[c] Predicted with the mean precipitation time using *in vitro* dissolution/precipitation test

[d] Results obtained from dog studies

[e] Results obtained from primate studies

developed by fitting the corresponding concentration-time profiles for each dog cohort with an optimized MPT. The obtained MPT values from the dog models were directly used in the human model to simulate the absorption under corresponding dosing scenarios. The human food effect was studied in healthy volunteers in a two-period, open label, single dose, cross-over clinical trial for both formulations. In two study periods, subjects were randomized to receive a single 200 mg dose under fasted and fed condition. Absorption modeling was performed for each formulation under fasted or fed conditions.

Case Study 5: Negative Food Effect for a BCS/BDDCS Class 3 Drug

NVS001 is a moderate to strong base (pKa=8.4) with a relatively low lipophilicity at physiological pH (log $D_{pH=7.4}$= 1.01) (Table II). NVS001 has a high water solubility and low Caco-2 permeability (Tables I and II). NVS001 is a high affinity (K_m: 3 μM) and moderate-capacity (J_{max}: 29×10^{-5} nmol/min·cm^2) substrate for the p-glycoprotein and is transported by organic anion-transporting peptide OATP2B1 in HEK293 cells with an estimated K_m of 72 μM and a maximum uptake clearance of 0.5 μL/min/mg protein. The majority of the absorbed oral dose was eliminated unchanged in the feces (77.7%) (Table I). NVS001 was orally administrated to fasted and non-fasted primates and food reduced C_{max} and $AUC_{(0-\infty)}$ by 92% and 85%, respectively (Table III, Fig. s2). Since NVS001 had a low permeability, both *via in silico* calculated P_{eff} (Table I) and P_{eff} derived from *in vitro* Caco-2 data (Table II), the intestinal uptake transporter OATP2B1 likely played a major role in the absorption. However, the organic anion transporting peptide was likely inhibited by food, as there was an over 85% reduction of the systemic exposure in non-fasted primates. Therefore, the kinetic data of uptake transporters were incorporated in the human model. The passive permeability data and p-glycoprotein kinetic data were included in both fasted and fed model. A single oral dose (300 mg) under fasted or fed state was given to healthy volunteers in a two-way and cross-over study. The human oral PK profile for the fasted state was first simulated using the GastroPlus ACAT model. The measured values of J_{max} and K_m of P-gp and OATP2B1 were used as inputs. The relative physiological distribution of P-gp and OATP2B1 (Table s1) have been reported previously (38) and were used in the ACAT model. However, the relative expression levels of OATP-2B1 between *in vitro* HEK293 cells and *in vivo* enterocytes remain unknown. The *in vitro*-to-*in vivo* scaling factor of K_m was set as 1.0, assuming that the substrate's affinity to the transporter are equivalent between *in vitro* expressed cells and *in vivo* enterocytes. The scaling factor for J_{max} of influx transporter of OATP2B1 was then fitted against the observed plasma concentration-time curves to account for the unknown ratio of the expression level. Further, the mechanism-based analysis of human oral PK profile under fed state was performed, assuming that the inhibitory interactions on OATP2B1 between food and NVS001 are competitive. Therefore, the apparent K_m of OATP2B1-mediated uptake of NVS001 in the gut tended to increase under fed state. A parameter optimization was performed to

identify the best scaling factor for K_m to describe the PK profile and match the AUC under fed state conditions.

Case Study 6: A priori Prediction of Food Effects for BCS/BDDCS Class 4 Drugs

NVS169 and NVS113 are both ampholytic compounds. Their aqueous solubility was low over the pH range of 1–8 for unformulated drug substance. Both compounds are substrates of P-gp and exhibited a low permeability (Table II). This suggested a risk for limited oral absorption and an uncertain direction for the food effect. To mitigate these dual challenges caused by limited solubility and permeability, a microemulsion formulation (ME) was designed for NVS169. An optimized solid formulation (SF) dosage form was designed for NVS113.

For NVS169, a ME formulation was initially tested in rats after oral dosing (1–200 mg/kg) under fasted conditions. For NVS113, a SF formulation was evaluated in the canine food effect model similar to case study 4. Simcyp ADAM or GastroPlus ACAT models were developed for rat or dog profiles to identify the *in vivo* solubility and/or permeability that best captured the concentration-time profiles. The optimized values were incorporated in a human model to simulate PK parameters and profiles after a single oral dose of 100 mg NVS169 or 30 mg NVS113 under fasted and fed condition *a priori* (Table III). Once clinical food effect profile data were available, they were used for assess the accuracy of the simulations.

RESULTS

Prospective predictions were performed to estimate the magnitude of food effects for most of compounds (Table III). For NVS001, retrospective modeling had to be done due to the unknown relative expression of uptake transporters. For NVS562, the model was further refined by fitting the parameter of MPT to observed clinical data to drive future clinical studies. The magnitudes of predicted food effects (represented as fold change of AUC and C_{max}) were compared with observed clinical results. (Table III). Overall, the fold changes of AUC and C_{max} (fed *vs.* fasted) estimated by current models for all of case studies fell within 1.6-fold of the observed values, indicating that the current modeling approach can accurately analyze food effects across BCS/BDDCS Class Compounds.

Case Example 1: NVS732

Initially, based on *in silico* data, NVS732 was classified as a pBCS/pBDDCS class 3 drug (Table I) due to its low calculated human P_{eff} (Table I), its low to moderate Caco-2 permeability and its high aqueous solubility (Table I and II). In the rat *in vivo*, however, F_a was high, indicating near complete absorption (87%) (Table I). Thus, NVS732 is an example where low *in vitro* Caco-2 permeability does not always result in low absorption *in vivo* (Table I) (39). Rat ADME data confirmed NVS732 as an rBCS/rBDDCS class 1 drug since absorption was high, and metabolism was extensive (64.3%). In human, NVS732 demonstrated hBCS/hBDDCS class 1 drug behavior (Tables I and III). A high-fat meal did not affect the extent of absorption or AUC (Fig. 3). While C_{max} was reduced by 19% and T_{max} was delayed by approximately 1 h, this decrease was not considered clinically relevant. The predicted plasma concentration *versus* time curve and PK parameters were in agreement with observed data (Fig. 3). The GastroPlus model successfully predicted bioequivalent exposure under fasted and fed dosage regimen (Table III). Predicted and observed results were consistent with the BCS/BDDCS, which stipulates that Class 1 drugs generally do not show any food effects (1, 7).

Case Example 2: NVS406

Based on *in silico* calculations, NVS406 was classified as a pBCS/pBDDCS Class 2 drug (Table I). NVS406 solubility was low in fasted state (0.004 mg/mL in FaSSIF) and fed state (0.1 mg/mL in FeSSIF). Rat ADME data confirmed NVS406 as a rBCS/rBDDCS Class 2 drug, as metabolism was >90% (Table I), with moderate absorption (45%) likely limited by low GI solubility. In human, NVS406 showed a significant positive food effect; which is consistent with hBCS/BDDCS Class 2 drugs (Table I and III). The mean of AUC and C_{max} increased by ~4.4 or ~4.3-fold and 6.1 or 6.1-fold under fed state conditions after a single dose of 150 mg or 450 mg of an oral coarse suspension, respectively (Table III). The magnitude of this food effect and plasma concentration profiles were well predicted when compared with observed data (Fig. 4, Table III). For the cohort receiving a 450 mg dose without food, the initial absorption and distribution phase from 0 to 24 h was well captured by the model, but the concentrations after 24 h of dose were underestimated (Fig. 4b). Overall, the predicted magnitude of food effect was still in good agreement with the observed results. Prediction fold-errors were less than 1.6 for AUC and 1.3 for C_{max}, respectively (Table III). In the fasted state, a flattened terminal concentration-time curve was observed, and the concentration after 12 h of the dose appeared to remain constant for up to 48 h and then declined more rapidly. One explanation may be a prolonged and slow absorption in the colon. According to the regional absorption plot (Fig. 4c), colonic absorption from the caecum and ascending colon accounted for ~45% of the total dose absorbed in the fasted state. However, the significance of

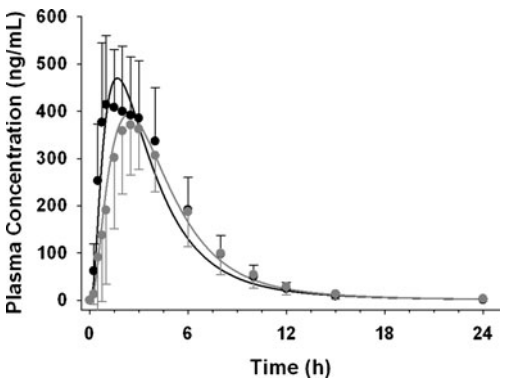


Fig. 3. Observed plasma concentrations are shown in *solid circles* (*black* fasted, *gray* fed). Simulated PK profile are presented as *solid curves* (*black* fasted, *gray* fed) after a single oral dose of 100 mg NVS732 in healthy volunteers

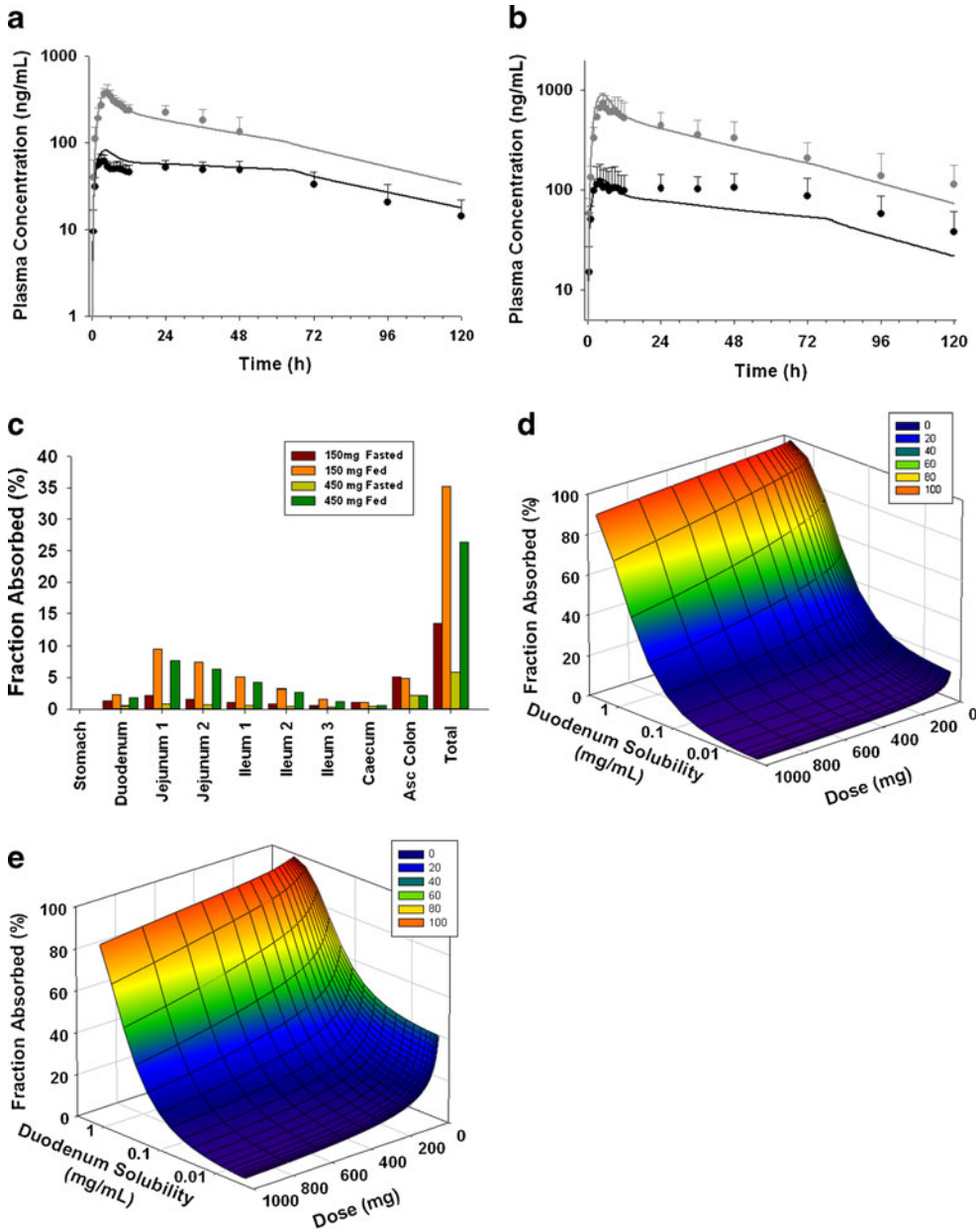


Fig. 4. Observed plasma concentrations of NVS406 are shown in *solid circles* (*black* fasted, *gray* fed). Simulated PK profile are presented as solid curves (*black* fasted, *gray* fed) after a single administration of **a** 150 mg of NVS406 and **b** 450 mg of NVS406 given as an oral suspension in healthy volunteers. **c** Fraction of regional absorption for NVS406 after a 150 or 450 mg dose in fasted and fed healthy volunteers. Surface response plot of the change of fraction absorbed (F_a) with respect to duodenum solubility (representing *in vivo* solubility) and dose under **d** fasted or **e** fed condition

the colonic absorption was attenuated in the fed state and only accounted for less than 13% of the total absorbed dose. The main absorption area under fed state located in the upper intestine where sufficient drug is available as a result of improved *in vivo* solubility, whereas the extent of colonic absorption was not pronounced due to the poor aqueous solubility. To design a formulation that could mitigate the positive food effect, it was important to explore the interaction between solubility and dose and their impacts on the extent of absorption under both fasted and fed conditions. A parameters analysis (PSA) was conducted under fasted and fed condition and three-dimension surface response plots (Fig. 4d, e) elucidated that F_a has a steep drop from ~80% to ~30% when duodenal solubility decreased from 1 mg/mL to 0.1 mg/mL under both fasted and fed states. F_a gradually decreased when doses were increased from 200 to 1,000 mg. This information was provided to guide formulation development of a new dosage form that could significantly enhance solubility along the gastrointestinal tract. To this end, a new liquid formulation and a coarse suspension were tested in the dog food effect model using a single oral dose of 5 mg/kg (equivalent to a ~190 mg dose in 70 kg adult). Significant differences for mean AUC and C_{max} values were not observed for the liquid formulation between fasted and fed

condition whereas a strong positive food effect (> 4.5 fold increase on AUC and C_{max}) was observed for the coarse suspensions (Table III). This suggested that the utility of a lipid-based formulation appeared to be an effective strategy to boost dissolution/solubility and enhance the oral bioavailability of a poorly water soluble compound under the fasted state.

Case Example 3: NVS562

NVS562 was classified between pBCS/rBDDCS classes 2–4 due to a low aqueous solubility and a moderate permeability (Table I). For NVS562, the *in silico* human P_{eff} was high (Table I), while the measured Caco-2 permeability was low leading to a low derived human P_{eff} permeability of 0.95×10^{-4}cm/s which less than that for metoprolol (1.8×10^{-4} cm/s) (Table II). As rat *in vivo* metabolism data were not available, NVS562 was still classified as rBCS/rBDDCS class 2 drug as rat *in vivo* absorption was high (> 80%) (Table I, Fig. 1).

The biorelevant solubility values for SEDDS of the free base and tablet formulations with the salt were listed in Table II. For NVS562, human food effects were formulation dependent (Table III). With SEEDS capsules, a high-fat meal did not affect the extent of absorption and the AUC values were unchanged. The geometric mean of C_{max} was reduced by 15% and T_{max} was delayed by approximately 3 h in the fed state. However, with a tablet formulation, the geometric mean of AUC and C_{max} increased by 1.6 and 1.6 fold and T_{max} was delayed by approximately 2 h (Table III). The *in vitro* two-step dissolution results are shown in Fig. 5a. Apparently supersaturation was maintained for over 2 h with the SEDDS formulation in FaSSIF and FeSSIF media, thus a high fat meal caused only negligible effects on drug dissolution and precipitation. The SEDDS formulation likely enhanced NVS562 solubility in the intestine, thus imparting desirable hBCS/BDDCS class 1 characteristics with minimal food effects on drug exposure. On the contrary, the tablet formulation did not mitigate positive food effects (Table III) likely due to precipitation under fasted and fed state, which was identified by the two-step dissolution test. The MPT values obtained from the *in vitro* and *in vivo* method as well as the AUC values predicted were summarized in Table s2. The plasma concentration time curves simulated by the model using *in vivo* optimized MPT were represented in Fig. 5b and c. For the SEDDS formulations under fasted and fed condition as well as a tablet formulation under fed condition, a model with *in vitro* MPT values (3,200–3,500 s) resulted in acceptable prediction PK profiles (data not shown) for AUC. A minor improvement of the simulation accuracy was achieved when MPT values were approximately doubled (e.g. 6,000 s; Fig. 5b). For the tablet formulation in the fasted state, the *in vitro* MPT (2,210 s, Table s2) slightly underestimated the extent of absorption. An MPT of 3,500 s best described the observed PK profile (Fig. 5c). Although the AUC value was slightly under predicted with the tablet formulation in the fasted state, the food effect trend was well predicted for both SEDDS and tablet formulations using *in vitro* MPT values (Table s2). A parameter analysis (PSA) for F_a change with respective to MPT is shown in Fig. 5d. MPT has less influence on F_a for SEDDS as solubility of NVS562 is sufficient, irrespective of pH and meal conditions. In such scenarios, the extent of absorption was always nearly complete (> 80%) and thereby bias of food effects prediction was generally minor using the PBPK model. On the other hand, for suspensions where solubility of NVS562 is limited, slower precipitation rate (or long MPT) prolonged the "supersaturation" state of compounds and likely allowed higher extent of absorption, suggesting that MPT is potentially a key driver for F_a in suspensions. Thus, inaccurate estimation of MPT can result in a large bias towards the prediction of food effects.

Case Example 4: NVS701

NVS701 was identified as a pBCS/pBDDCS class 2 to 4 due to a low aqueous solubility, and a moderate permeability (Table I). Rat ADME data confirmed NVS701 as an rBCS/rBDDCS 2 drug due to extensive metabolism (84.7%) and low absorption. In human, NVS701 demonstrated hBCS/hBDDCS class 2 drug behavior (Table I) and a positive food effect (Table III). A high-fat meal increased the extent of absorption following the administration of Capsules F1 and Capsule F2 similarly in dogs and humans (Table III). For both formulations, the values of MPT optimized by fitting plasma concentration-time curves under fed state were higher than the values under fasted conditions, suggesting that solubilized NVS701 can remain in a supersaturated state in the intestine for a longer time after high fat meals. The simulated results were also in agreement with the fact that meals can stimulate bile flow and enhance solubility of poorly water soluble compound in small intestine (7, 40–42). The resulting human model captured the mean observed data reasonably well in fasted and fed conditions for both formulations using the MPT obtained from the optimized dog model (Fig. 6). The predicted magnitude of food effects in human in terms of changes on AUC and C_{max} was less than 1.1 fold of the observed effects (Table III).

Case Example 5: NVS001

Based on *in silico* and early discovery data, NVS001 was classified as a pBCS/pBDDCS Class 3 drug due to its high solubility and low permeability (Table I). In the rat, metabolism was low (17.3%), thus NVS001 was classified as a pBCS/pBDDCS class 3 drug. In human, NVS001 also demonstrated hBCS/hBDDCS class 3 behaviors. Food greatly reduced NVS001 C_{max} by 72% and $AUC_{0-\infty}$ by 69% (Table III). To best describe the concentration-time curve of NVS001 in the fasted state, an *in vitro-in vivo* scaling factor for J_{max} of 0.055 was fitted against the data, suggesting that the expression level of OATP2B1 in the gut are lower than those in the *in vitro* expressed HEK293 cells. For oral absorption modeling, the same scaling factor for J_{max} was used in the fasted and fed state since transporter expression level was constant between fasted and fed conditions. On the other hand, a scaling factor for K_m was estimated to be 50 to fit the concentration-time curve (Fig. 7). The fitted K_m scaling factor in the fed state implied that the food components competitively inhibited the uptake transporter of OATP2B1 and resulted in reduced NVS001 absorption. Overall, the

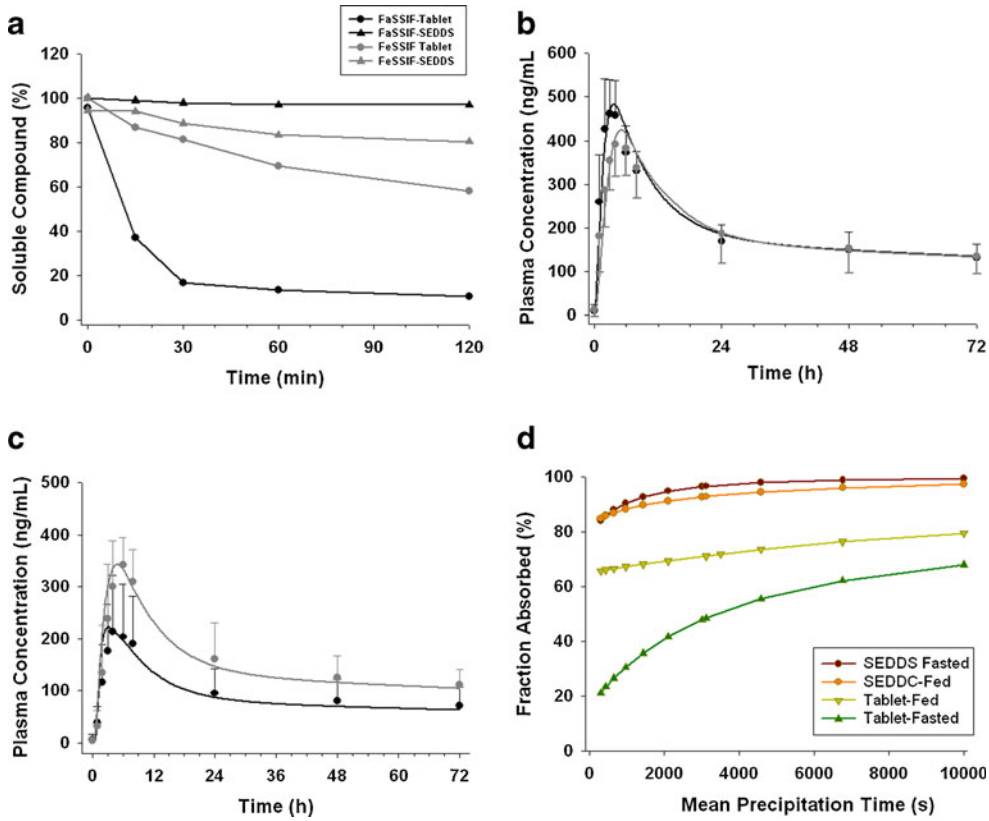


Fig. 5. **a** *In vitro* precipitation profiles of two formulations containing NVS562 in FaSSIF or FeSSIF media. Observed plasma concentrations are shown in *solid circles* (*black* fasted, *gray* fed) and simulated PK profile are presented as *solid curves* (*black* fasted, *gray* fed) after a single administration of 150 mg of NVS562 given as a **b** SEDDS or **c** tablet in healthy volunteers. **d** Parameter sensitivity of precipitation time to the change of fraction absorbed

observed negative food effect was consistent with the predicted results obtained with the ACAT model (Table III).

Case Example 6: NVS169 and NVS113

NVS169 and NVS113 were identified as borderline pBCS/pBDDCS class 4 and 2 compounds based on *in silico* prediction, respectively (Table I) with low solubility and low permeability, but high clogP. The *in vitro* measured solubility for NVS113 was even lower than the *in silico* predicted solubility (Tables I and II). The rat ADME study showed metabolism higher than 60% for both NVS113 (65%) and NVS169 (81.3%) (Table I), thus resulting in an rBCS/rBDDCS class 2. During clinical development, formulation efforts greatly increased the solubility/dissolution for both drug substances (Table II). For NVS169, the fraction absorbed in rats exceeded 80% with an ME formulation, suggesting that absorption was not limited by solubility. The values of P_{eff} and solubility that can best describe the rat PK profiles were 1.78×10^{-4}cm/s and 0.78 mg/mL. Using these values in the human model, no changes in AUC and C_{max} with or without meals were predicted. The predictions were partly in agreement with the clinical observations, where AUC was not significantly altered, but C_{max} decreased by 40% (Table III). For NVS113, no food effect was observed in canine model. Similarly, *in vivo* solubility values were obtained and used in human PBPK model. The human model predicted an insignificant food effect with less than 10% decrease of AUC and C_{max} when comparing PK data under fed and fasted state. However, the observed clinical data showed a more significant reduction of AUC (~30%) in the fed state (Table III). The reasons are not entirely understood, but may be due to an unknown drug complexation with food components or possible transporter interactions.

DISCUSSIONS

Food can induce changes in physiological conditions, such as delayed in gastric emptying, change of gastrointestinal pH, stimulation of bile flow, and interaction of intestinal influx or efflux transporters (1, 2, 8, 9). A positive food effect is manifested by a higher systemic exposure when the drug is given with food compared to the fasted state and mainly seen for BCS/BDDCS 2 drugs. A negative food effect is manifested by a reduced exposure when the drug is given with food and is mainly seen for BCS/BDDCS 3 drugs. BCS/BDDCS Class 1 drugs often show no food effect. For BCS/BDDCS 4 drugs food effects is often difficult to predict, as they can be dose and formulation dependent. Presence of a food effect can impact drug labeling (2) and convenience to the patient. Briefly, a food effect is observed if the 90%

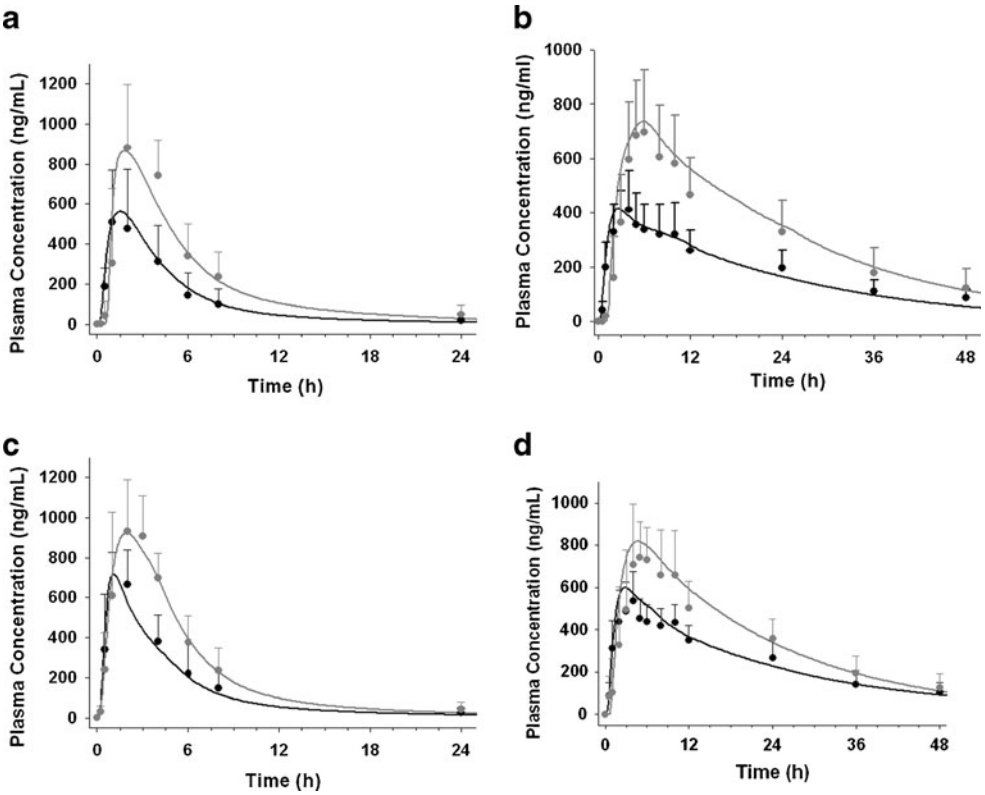


Fig. 6. Observed plasma concentrations are shown in *solid circles* (*black* fasted, *gray* fed) and simulated PK profile are presented as *solid curves* (*black* fasted, *gray* fed) after a single administration of 50 mg of NVS701 given as a **a** marketed capsule (capsule F1) or **c** solid suspended microemulsion (capsule F2) in dogs or 200 mg of NVS701 given as a **b** capsule F1 or **d** capsule F2 in healthy volunteers

confidence interval for the ratio of population geometric means between fed and fasted treatments fails to meet the limits of 80–125% for either AUC or C_{max} (2).

Potential human food effects can be predicted to aid product or formulation development (43) at all drug development stages using *in silico*, *in vitro,* and *in vivo* methods as shown in Fig. 1. Here, both BCS and BDDCS classifications (1, 7) aided in identifying likely food effects for seven proprietary compounds from early discovery through early development using readily available data (Fig. 1, Table I).

For early discovery, a preliminary pBCS/pBDDCS class is established for a NCE when little or no *in vivo* ADME properties are available. To establish a pBCS, key *in silico* parameters (e.g. human effective permeability, and intrinsic solubility) can be calculated using e.g. the GastroPlus ADMET predictor. Alternative *in silico* tools are available (e.g. ALOGPS, VolSurf, *etc.*) to calculate these and related physicochemical parameters. As shown in Fig. 1, we assigned a preliminary BCS class of NCEs based on a calculated intrinsic solubility and a human effective permeability (P_{eff}).

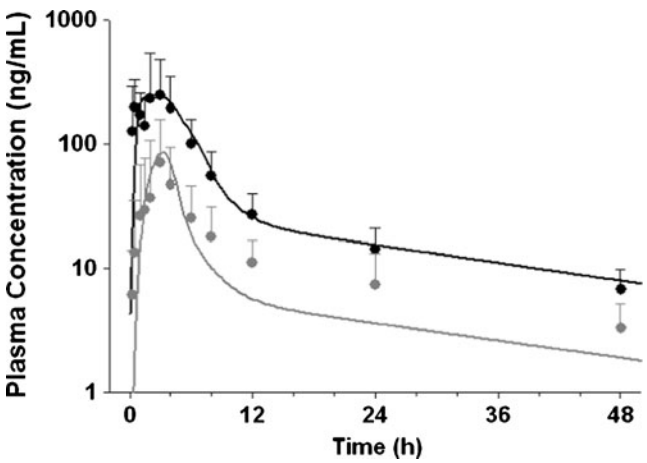


Fig. 7. Observed plasma concentrations are shown in *solid circles* (*black* fasted, *gray* fed) and simulated PK profile are presented as *solid curves* (*black* fasted, *gray* fed) after a single administration of 300 mg of compound NVS001 given as a tablet in healthy volunteers

A differentiating solubility value of 0.5 mg/mL was arbitrarily chosen; as this concentration is achieved for a 125 mg efficacious dose given with 250 mL water. For permeability assessment, metoprolol was chosen as a high permeability reference compound (44, 45) (Tables I and II). Calculated LogP (cLogP) values were used to describe the probability of extensive metabolism. This was done retrospectively, as this approach was only recently published (10). Compounds with a cLogP value greater than 2, are likely to be eliminated mainly *via* metabolism and can be assigned to Class 1 or Class 2 in the pBDDCS (10). Our practical preliminary pBCS/pBDDCS classes could thus identify food effect risks early and guide a formulation strategy of the NCE during later development stages.

During the candidate selection stage, rat *in vivo* mass balance/disposition data from radiolabeled drug substances allowed estimation of F_a and extent of metabolism (46) leading to a rat based BCS/BDDCS or rBCS/rBDDCS (Fig. 1). High metabolism or extensive metabolism were defined if >60% of the drug was metabolized after intravenous dosing and resulted in an rBDDCS class 1 or 2, depending on solubility, F, or F_a. The rBCS/rBDDCS was used to confirm or complement the pBCS/pBDDCS, derived *via* the earlier *in silico* approach. For the seven Novartis compounds, there was a general agreement with the food effects that could be expected from the rBCS/rBDDCS and pBCS/pBDDCS when compared to observed food effects in the clinic (Table I). Thus, overall, the pBCS/pBDDCS and rBCS/rBDDCS classes predicted the likely direction clinical food effects.

In early clinical development, quantitative food effect predictions along with exposure profiles are typically desired by project teams, and often more than one formulation is under consideration for human trials. Advanced PBPK modeling tools readily allow the mechanism-based simulation of concentration-time profiles for liquid or solid dosage forms both in the fasted and fed states using physiological based absorption model and input parameters such as physicochemical properties and biorelevant solubility (33). As shown in Fig. 2, human systemic PK parameter (e.g., V_{ss}, CL), could be scaled from preclinical data using, e.g., the latest PhRMA scaling methods (47, 48) when human IV data were not available. *In vivo* relevant biopharmaceutical properties, some of which have not been extensively discussed in the literature, i.e., drug precipitation, transporter interactions, and formulation effects were included our absorption models. Here, we provide case examples and potential solutions on how to generate *in vivo* precipitation and solubility data toward human food effect profile predictions. We used *in vivo* observed dog data to describe an ACAT model and *in vivo* solubility/precipitation data can be obtained by fitting the observed dog exposure profiles (cases NVS701, NVS562). These data were then used for human profile predictions (shown schematically in Fig. 2). Food effects can be formulation dependent and can sometimes be mitigated with optimized formulations (case NVS562). We also analyzed transporter involvement in food effects for a BCS/BDDCS class 3 compound (case NVS001). The impacts of formulation on the food effects of BCS class 4 compounds were studied with prospective predictions (NVS169, NVS113).

For weak bases, precipitation can limit oral exposure both in fasted and fed states (1). To model *in vivo* precipitation, an MPT parameter was used to represent the time of supersaturation under fasted and fed conditions. We proposed two methods for this parameter estimation: (1) calculating the AUMC/AUC ratio based on drug precipitation profiles obtained from a two-step biorelevant dissolution test or (2) fitting this parameter against the observed PK profiles in dogs under fasted and fed conditions. For NVS562, the simulation results showed that the parameter of MPT obtained from the two-step biorelevant dissolution test underestimated the human plasma concentration after a single suspension dose under fasted state. A donor-to-acceptor transfer model has been reported to simulate the slow transfer process for poorly soluble weak bases from stomach to intestine, which may potentially better predict MPT (34). However, this approach has not been fully validated. Here, the human plasma concentration time profiles of NVS701 were well described using the MPT obtained from fitting dog PK data under fasted or fed condition. Dog is the most studied species for predicting human food effects (12–14) as clinical service forms (e.g., capsule or tablet) can be directly administered to dogs. For NVS701, the dog model was not only used as a surrogate human food effect model but was a useful tool to generate the MPT in human models. To our knowledge, no prior attempts have been published to successfully apply optimized MPT data from canine model to quantitatively predict the human food effects. Yet, the prediction accuracy using such an approach may depend on the compound properties and formulations. It was critical that human relevant disposition parameters and physicochemical properties are used. If the MPT parameter is found to be inadequate and not translatable between dog and human, then the model may have to be refined when observed human PK data become available.

Transporter interactions can be important for BCS class 3 compounds (7). *A priori* predictions of negative food effects caused by food and intestinal influx transporter interactions can be difficult as inhibitory effects of dietary substances on transporter cannot be measured. A retrospective analysis demonstrated the impact of food on intestinal transporter inhibition by optimizing the value of an *in vitro* to *in vivo* scaling factor for K_m for the related uptake transporters. If absorption of BCS class 3 compounds is mediated by an intestinal influx transporter, high-fat meals will decrease the extent of absorption due to inhibition or competition of uptake transporters in the intestine. Wu and Benet had noted that the overall exposure changes with class 3 compounds depend upon whether meals have more pronounced effects on the efflux or influx transporters in the absorption process. An unexpected increase or minimal meal effect in the extent of absorption can be observed (e.g., acyclovir). Due to the complexity of food and transporter interaction, a preclinical model may provide a useful insight into the direction of food effects. For NVS001, the food effects observed in primates were similar to those observed in human with over 85% reduction in AUC (Fig. s2 and Fig. 7). Such results can serve as an important translatable link for establishing mechanism-based human PBPK models.

The prediction of clinical food effects for BCS/BDDCS class 2 to 4 compounds (both of which can exhibit Class 4 behavior at higher doses or with suboptimal formulations) can be very challenging. We successfully predicted the lack of

positive food effects for two BCS/BDDCS class 2 to 4 compounds (Table III). NVS169 was extensively metabolized and was likely not a substrate for absorptive transporters, similar to BCS Class 1 and 2 compounds (7). Apparently low solubility can be a rate limiting factor in NVS169 oral absorption. The calculated dose number at 100 mg for the drug substance (DS) was 3.1, which was successfully reduced to 0.51 with a microemulsion (ME) formulation (Tables I and II). Thus, solubility is currently not rate-limiting step for drug absorption. This represented a successful example where formulations can change a compound's BCS Class and a reduced risk for food effects (as was also shown for NVS562). For NVS113, also no positive food effect had been predicted (Table III). Interestingly, a significant negative food effect was observed in the clinic—a trend which had not been identified in the dog model (Table III). Thus for NVS113, as with other Class 4 drugs, food effects are apparently mediated by factors other than solubility and which can include drug-food complexation or uptake transporter interactions. It is likely that absorptive intestinal transporters were involved in the absorption process and food may inhibit the drug uptake process, resulting in a negative food effects. Taken together, these two examples indicated the complexity in prediction and mitigation of food effects for BCS Class 4 compounds. It further illustrated that a compounds' BCS/BDDCS class can be formulation-dependent.

CONCLUSIONS

Integration of *in silico*, *in vitro*, and preclinical *in vivo* data with ACAT PBPK models, allowed early identification of likely food effect risks and exposure profile predictions across BCS/BDDCS class 1 through 4 compounds. A practical preclinical BCS/BDDCS, which relies mainly on calculated parameters, could be readily implemented by DMPK or formulation scientists for all drug development stages. Rat *in vivo* absorption and metabolism data aided in identifying or confirming the likely human BDDCS class. Various *in vitro* and *in vivo* preclinical tools were successfully integrated with commercially available software to conduct physiologically based human absorption and exposure modeling for orally-dosed compounds with or without meals. Formulation-dependent food effects, once understood, could sometimes be solved with optimized formulations. Overall, integrated and practical approaches provided representative case examples for food effect predictions and risk identification prior to clinical studies. Yet, challenges for food effect predictions do remain, especially for BCS/BDDCS class 3 and 4 drugs due to insufficient knowledge of *in vivo* intestinal transporter expression levels, meal effects on intestinal transporters, and complex food-drug interactions.

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Conflict of Interests None.

REFERENCES

1. Fleisher D, Li C, Zhou Y, Pao LH, Karim A. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. Clin Pharmacokinet. 1999;36(3):233–54.
2. US FDA. Food–effect bioavailability and fed bioequivalence studies. In: Guidance for industry. http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm126833.pdf. 2002. Accessed 02 Jun 2012.
3. Zhang X, Lionberger RA, Davit BM, Yu LX. Utility of physiologically based absorption modeling in implementing quality by design in drug development. AAPS J. 2011;13(1):59–71. doi:10.1208/s12248-010-9250-9.
4. Hendeles L, Weinberger M, Milavetz G, Hill 3rd M, Vaughan L. Food-induced "dose-dumping" from a once-a-day theophylline product as a cause of theophylline toxicity. Chest. 1985;87(6):758–65.
5. Wilder BJ, Leppik I, Hietpas TJ, Cloyd JC, Randinitis EJ, Cook J. Effect of food on absorption of dilantin kapseals and mylan extended phenytoin sodium capsules. Neurology. 2001;57(4):582–9.
6. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification—the correlation of *in-vitro* drug product dissolution and *in-vivo* bioavailability. Pharmaceut Res. 1995;12(3):413–20.
7. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res. 2005;22(1):11–23. doi:10.1007/s11095-004-9004-4.
8. Benet L. Z. WCY. Using a biopharmaceutics drug disposition classification system to predict bioavailability and elimination characteristics of new molecular entities. Somerset, NJ: NJDMDG. 2006.
9. Custodio JM, Wu C-Y, Benet Leslie Z. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. Adv Drug Deliv Rev. 2008;60(6):717–33.
10. Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. AAPS J. 2011;13(4):519–47. doi:10.1208/s12248-011-9290-9.
11. Singh A, Worku ZA, Van den Mooter G. Oral formulation strategies to improve solubility of poorly water-soluble drugs. Expert Opin Drug Deliv. 2011;8(10):1361–78. doi:10.1517/17425247.2011.606808.
12. Lui CY, Amidon GL, Berardi RR, Fleisher D, Youngberg C, Dressman JB. Comparison of gastrointestinal Ph in dogs and humans—implications on the use of the beagle dog as a model for oral absorption in humans. J Pharm Sci. 1986;75(3):271–4.
13. Meyer JH, Dressman J, Fink A, Amidon G. Effect of size and density on canine gastric-emptying of nondigestible solids. Gastroenterology. 1985;89(4):805–13.
14. Akimoto M, Nagahata N, Furuya A, Fukushima K, Higuchi S, Suwa T. Gastric pH profiles of beagle dogs and their use as an alternative to human testing. Eur J Pharm Biopharm. 2000;49(2):99–102.
15. Lentz KA, Quitko M, Morgan DG, Grace JE. Development and validation of a preclinical food effect model. J Pharm Sci. 2007;96(2):459–72. doi:10.1002/Jps.20767.
16. Russell WMS, Burch RL. The principles of humane experimental technique. London: Methuen & Co. Special edition published by Universities Federation for Animal Welfare (UFAW), 1992; 1959.
17. Huang SM. PBPK as a tool in regulatory review. Biopharm Drug Dispos. 2012;33(2):51–2. doi:10.1002/Bdd.1777.
18. Lukacova V, Woltosz WS, Bolger MB. Prediction of modified release pharmacokinetics and pharmacodynamics from *in vitro*, immediate release, and intravenous data. AAPS J. 2009;11(2):323–34. doi:10.1208/s12248-009-9107-2.

19. Parrott N, Lukacova V, Fraczkiewicz G, Bolger MB. Predicting pharmacokinetics of drugs using physiologically based modeling—application to food effects. AAPS J. 2009;11(1):45–53. doi:10.1208/s12248-008-9079-7.
20. Vieira MLT, Zhao P, Berglund EG, Reynolds KS, Zhang L, Lesko LJ, *et al*. Predicting drug interaction potential with a physiologically based pharmacokinetic model: a case study of telithromycin, a time-dependent CYP3A inhibitor. Clin Pharmacol Ther. 2012;91(4):700–8. doi:10.1038/clpt.2011.305.
21. Shaffer CL, Scialis RJ, Rong HJ, Obach RS. Using Simcyp to project human oral pharmacokinetic variability in early drug research to mitigate mechanism-based adverse events. Biopharm Drug Dispos. 2012;33(2):72–84. doi:10.1002/Bdd.1768.
22. Shono Y, Jantratid E, Dressman JB. Precipitation in the small intestine may play a more important role in the *in vivo* performance of poorly soluble weak bases in the fasted state: case example nelfinavir. Eur J Pharm Biopharm. 2011;79(2):349–56. doi:10.1016/j.ejpb.2011.04.005.
23. Shono Y, Jantratid E, Janssen N, Kesisoglou F, Mao Y, Vertzoni M, *et al*. Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling. Eur J Pharm Biopharm. 2009;73(1):107–14. doi:10.1016/j.ejpb.2009.05.009.
24. Shono Y, Jantratid E, Kesisoglou F, Reppas C, Dressman JB. Forecasting *in vivo* oral absorption and food effect of micronized and nanosized aprepitant formulations in humans. Eur J Pharm Biopharm. 2010;76(1):95–104. doi:10.1016/j.ejpb.2010.05.009.
25. Nicolaides E, Symillides M, Dressman JB, Reppas C. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. Pharm Res. 2001;18(3):380–8.
26. Dressman JB, Reppas C. *In vitro-in vivo* correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci. 2000;11:S73–80.
27. Parrott N, Lave T. Prediction of intestinal absorption: comparative assessment of GASTROPLUS (TM) and IDEA (TM). Eur J Pharm Sci. 2002;17(1–2):51–61.
28. Kuentz M, Nick S, Parrott N, Rothlisberger D. A strategy for preclinical formulation development using GastroPlus (TM) as pharmacokinetic simulation tool and a statistical screening design applied to a dog study. Eur J Pharm Sci. 2006;27(1):91–9. doi:10.1016/j.ejps.2005.08.011.
29. Yu LX, Amidon GL. Characterization of small intestinal transit time distribution in humans. Int J Pharm. 1998;171(2):157–63.
30. Heimbach T, Lakshminarayana SB, Hu WY, He HD. Practical anticipation of human efficacious doses and pharmacokinetics using *in vitro* and preclinical *in vivo* Data. AAPS J. 2009;11(3):602–14. doi:10.1208/s12248-009-9136-x.
31. Xia B, Heimbach T, Lin TH, He H, Wang Y, Tan E. Novel physiologically based pharmacokinetic modeling of patupilone for human pharmacokinetic predictions. Canc Chemother Pharmacol. 2012;69(6):1567–82. doi:10.1007/s00280-012-1863-5.
32. Kesisoglou F, Wu YH. Understanding the effect of API properties on bioavailability through absorption modeling. AAPS J. 2008;10(4):516–25. doi:10.1208/s12248-008-9061-4.
33. Jones HM, Parrott N, Ohlenbusch G, Lave T. Predicting pharmacokinetic food effects using biorelevant solubility media and physiologically based modelling. Clin Pharmacokinet. 2006;45(12):1213–26.
34. Kostewicz ES, Wunderlich M, Brauns U, Becker R, Bock T, Dressman JB. Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine. J Pharm Pharmacol. 2004;56(1):43–51. doi:10.1211/0022357022511.
35. De Buck SS, Sinha VK, Fenu LA, Nijsen MJ, Mackie CE, Gilissen RAHJ. Prediction of human pharmacokinetics using physiologically based modeling: a retrospective analysis of 26 clinically tested drugs. Drug Metab Dispos. 2007;35(10):1766–80. doi:10.1124/dmd.107.015644.
36. De Buck SS, Sinha VK, Fenu LA, Gilissen RA, Mackie CE, Nijsen MJ. The prediction of drug metabolism, tissue distribution, and bioavailability of 50 structurally diverse compounds in rat using mechanism-based absorption, distribution, and metabolism prediction tools. Drug Metab Dispos. 2007;35(4):649–59. doi:10.1124/dmd.106.014027.
37. Poulin P, Theil FP. Prediction of pharmacokinetics prior to in vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition. J Pharm Sci. 2002;91(5):1358–70. doi:10.1002/jps.10128.
38. Meier Y, Eloranta JJ, Darimont J, Ismair MG, Hiller C, Fried M, *et al*. Regional distribution of solute carrier mRNA expression along the human intestinal tract. Drug Metab Dispos. 2007;35(4):590–4. doi:10.1124/dmd.106.013342.
39. Chen ML, Yu L. The use of drug metabolism for prediction of intestinal permeability. Mol Pharmaceut. 2009;6(1):74–81. doi:10.1021/Mp8001864.
40. Mithani SD, Bakatselou V, TenHoor CN, Dressman JB. Estimation of the increase in solubility of drugs as a function of bile salt concentration. Pharmaceut Res. 1996;13(1):163–7.
41. Litman T, Druley TE, Stein WD, Bates SE. From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci. 2001;58(7):931–59.
42. Mithani SD, Bakatselou V, TenHoor CN, Dressman JB. Estimation of the increase in solubility of drugs as a function of bile salt concentration. Pharm Res. 1996;13(1):163–7.
43. Lentz KA. Current methods for predicting human food effect. AAPS J. 2008;10(2):282–8. doi:10.1208/s12248-008-9025-8.
44. US FDA. Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. In: Guidance for industry. 2000. http://www.fda.gov/downloads/Drugs/…/Guidances/ucm070246.pdf. Accessed 02 Jun 2012.
45. Fagerholm U, Johansson M, Lennernas H. Comparison between permeability coefficients in rat and human jejunum. Pharm Res. 1996;13(9):1336–42.
46. Tse FLS. Pharmacokinetics in drug discovery and development: nonclinical studies. In: Welling PG, Tse FLS, editors. Pharmacokinetics: regulatory, industrial, academic perspectives. 2nd ed. New York: Dekker; 1995. p. 300–6.
47. Jones RD, Jones HM, Rowland M, Gibson CR, Yates JW, Chien JY, *et al*. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 2: comparative assessment of prediction methods of human volume of distribution. J Pharm Sci. 2011. doi:10.1002/jps.22553.
48. Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones RD, *et al*. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: Comparative assessement of prediction methods of human clearance. J Pharm Sci. 2011. doi:10.1002/jps.22552.

EXHIBIT 4

US011426373B2

(12) **United States Patent**
Allphin et al.

(10) **Patent No.: US 11,426,373 B2**
(45) **Date of Patent: Aug. 30, 2022**

(54) **GAMMA-HYDROXYBUTYRATE COMPOSITIONS AND THEIR USE FOR THE TREATMENT OF DISORDERS**

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(52) **U.S. Cl.**
CPC ***A61K 31/19*** (2013.01); *A61K 2300/00* (2013.01)

(58) **Field of Classification Search**
CPC A61K 31/19; A61P 25/20
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,051,619	A	8/1962	Laborit
3,419,588	A	12/1968	De Man
4,221,778	A	9/1980	Raghunathan
4,374,441	A	2/1983	Carter et al.
4,393,236	A	7/1983	Klosa
4,510,128	A	4/1985	Khanna
4,524,217	A	6/1985	Davenport et al.
4,687,662	A	8/1987	Schobel
4,738,985	A	4/1988	Kluger et al.
4,916,161	A	4/1990	Patell
4,939,949	A	7/1990	Langenberg
4,983,632	A	1/1991	Gessa et al.
5,294,430	A	3/1994	Borch et al.
5,380,937	A	1/1995	Koehler et al.
5,415,870	A	5/1995	Gergely et al.
5,594,030	A	1/1997	Conte et al.
5,753,708	A	5/1998	Koehler et al.
5,758,095	A	5/1998	Albaum et al.
5,833,599	A	11/1998	Schrier et al.
5,840,331	A	11/1998	Van Cauter et al.
5,845,255	A	12/1998	Mayuad
5,955,106	A	9/1999	Moeckel et al.
5,990,162	A	11/1999	Sharf
6,014,631	A	1/2000	Teagarden et al.
6,022,562	A	2/2000	Autant et al.
6,067,524	A	5/2000	Byerly et al.
6,112,182	A	8/2000	Akers et al.
6,317,719	B1	11/2001	Schrier et al.
6,322,819	B1	11/2001	Burnside et al.
6,356,873	B1	3/2002	Teagarden et al.
6,384,020	B1	5/2002	Flanner et al.
6,436,998	B1	8/2002	Cacciaglia et al.
6,472,431	B2	10/2002	Cook et al.
6,472,432	B1	10/2002	Perricone
6,495,598	B1	12/2002	Yoneda et al.
6,565,872	B2	5/2003	Wu et al.
6,780,889	B2	8/2004	Cook et al.
7,015,200	B2	3/2006	Mamelak et al.
7,072,840	B1	7/2006	Mayuad
7,262,219	B2	8/2007	Cook et al.
7,568,822	B2	8/2009	Ibrahim
7,668,730	B2	2/2010	Reardan et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2 112663 C	4/2002
CA	2 510 289 A1	7/2004

(Continued)

OTHER PUBLICATIONS

"Guidance for Industry. Food-Effect Bioavailability and Fed Bioequivalence Studies." U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Dec. 2002. (Year: 2002).*

Borgen et al. (The Influence of Gender and Food on the Pharmacokinetics of Sodium Oxybate Oral Solution in Healthy Subjects. Journal of Clinical Pharmacology, 2003;43:59-65). (Year: 2003).*

"HIB-IMUNE," Physicians Desk Reference (41st ed.), (1987), 1095-1096.

"HibVAX," Physicians Desk Reference (41st ed.), (1987), 870.

"Phospholine Iodide," Physicians Desk Reference (50th ed.), (1996), 2784.

"Taxotere," Physicians Desk Reference (51st ed.), (1997), 2204-2207.

(Continued)

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(57) **ABSTRACT**

Provided herein are pharmaceutical compositions and formulations comprising mixed salts of gamma-hydroxybutyrate (GHB). Also provided herein are methods of making the pharmaceutical compositions and formulations, and methods of their use for the treatment of sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

11 Claims, 6 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,765,106	B2	7/2010	Reardan et al.
7,765,107	B2	7/2010	Reardan et al.
7,797,171	B2	9/2010	Reardan et al.
7,851,506	B2	12/2010	Cook et al.
7,895,059	B2	2/2011	Reardan et al.
8,101,209	B2	1/2012	Legrand et al.
8,193,211	B2	6/2012	Liang et al.
8,202,537	B2	6/2012	Mehta et al.
8,263,125	B2	9/2012	Vaya et al.
8,263,650	B2	9/2012	Cook et al.
8,324,275	B2	12/2012	Cook et al.
8,457,988	B1	6/2013	Reardan et al.
8,461,197	B2	6/2013	Tung
8,461,203	B2	6/2013	Cook et al.
8,529,954	B2	9/2013	Lebon et al.
8,589,182	B1	11/2013	Reardan et al.
8,591,922	B1	11/2013	Allphin et al.
8,598,191	B2	12/2013	Liang et al.
8,680,228	B2	3/2014	Guo et al.
8,731,963	B1	5/2014	Reardan et al.
8,759,394	B2	6/2014	Tung et al.
8,771,735	B2	7/2014	Rourke et al.
8,772,306	B1	7/2014	Eller
8,778,301	B2	7/2014	Mamelak et al.
8,778,398	B2	7/2014	Rourke et al.
8,859,619	B2	10/2014	Cook et al.
8,901,173	B2	12/2014	Allphin et al.
8,952,029	B2	2/2015	Eller
8,952,062	B2	2/2015	Cook et al.
9,023,400	B2	5/2015	Guimberteau et al.
9,050,302	B2	9/2015	Eller
9,132,107	B2	9/2015	Allphin et al.
9,486,426	B2	11/2016	Eller
9,539,330	B2	1/2017	Cook et al.
9,555,017	B2	1/2017	Allphin et al.
9,770,514	B2	9/2017	Ghebre-Sellassie
9,795,567	B2	10/2017	Rourke et al.
9,801,852	B2	10/2017	Allphin
10,195,168	B2	2/2019	Allphin et al.
10,213,400	B2	2/2019	Eller
10,272,062	B2	4/2019	Mégret et al.
10,398,662	B1	9/2019	Allphin et al.
10,736,866	B2	8/2020	Mégret et al.
10,758,488	B2	9/2020	Allphin et al.
10,813,885	B1	10/2020	Allphin et al.
10,925,844	B2	2/2021	Grassot et al.
10,952,986	B2	3/2021	Megret et al.
10,959,956	B2	3/2021	Allphin et al.
10,966,931	B2	4/2021	Allphin et al.
10,973,795	B2	4/2021	Megret et al.
10,987,310	B2	4/2021	Allphin et al.
11,077,079	B1	8/2021	Allphin et al.
11,090,269	B1	8/2021	Allphin et al.
2003/0180249	A1	9/2003	Khanna et al.
2004/0092455	A1	5/2004	Mamelak et al.
2005/0031688	A1	2/2005	Ayala
2005/0037077	A1	2/2005	Legrand et al.
2005/0113366	A1	5/2005	Bourguignon et al.
2005/0142192	A1	6/2005	Benjamin et al.
2006/0018933	A1	1/2006	Vaya et al.
2006/0024365	A1	2/2006	Vaya et al.
2006/0069040	A1	3/2006	Mamelak
2006/0210630	A1	9/2006	Liang et al.
2006/0228410	A1	10/2006	Dumont et al.
2007/0270491	A1	11/2007	Cook et al.
2008/0003267	A1	1/2008	Spencer et al.
2008/0069871	A1	3/2008	Vaughn et al.
2008/0085304	A1	4/2008	Baichwal et al.
2008/0118571	A1	5/2008	Lee et al.
2008/0226564	A1	9/2008	Weers et al.
2008/0292700	A1	11/2008	Nghiem et al.
2008/0293698	A1	11/2008	Johnson
2009/0137565	A1	5/2009	Frucht
2009/0155357	A1	6/2009	Muhuri
2009/0317355	A1	12/2009	Roth et al.
2010/0112056	A1	5/2010	Rourke et al.
2010/0266701	A1	10/2010	Guimberteau et al.
2011/0034727	A1	2/2011	Luchi et al.
2011/0039929	A1	2/2011	Cook et al.
2011/0091537	A1	4/2011	Castan et al.
2011/0111027	A1	5/2011	Rourke et al.
2011/0213004	A1	9/2011	Kim et al.
2012/0020833	A1	1/2012	Cook et al.
2012/0076865	A1	3/2012	Allphin et al.
2012/0148672	A1	6/2012	Mehta et al.
2012/0202879	A1	8/2012	Cook et al.
2012/0202880	A1	8/2012	Cook et al.
2013/0230587	A1	9/2013	Pilgaonkar et al.
2013/0273159	A1	10/2013	Howard et al.
2014/0004202	A1	1/2014	Suplie et al.
2014/0037745	A1	2/2014	Liang et al.
2014/0072624	A1	3/2014	Jung et al.
2014/0093578	A1	4/2014	Mehta et al.
2014/0127306	A1	5/2014	Mehta et al.
2014/0141090	A1	5/2014	Wilson
2014/0171506	A1	6/2014	Allphin et al.
2014/0271896	A1	9/2014	Abu Shmeis et al.
2014/0348917	A1	11/2014	Rourke et al.
2015/0005334	A1	1/2015	Shah et al.
2015/0073052	A1	3/2015	Cook et al.
2015/0328168	A1	11/2015	Daviaud-Venet et al.
2016/0068463	A1	3/2016	Peoples et al.
2016/0228379	A1	8/2016	Kumar et al.
2016/0271070	A1	9/2016	Singh et al.
2016/0338966	A1	11/2016	Guimberteau et al.
2016/0346200	A1	12/2016	Sommer et al.
2016/0346216	A1	12/2016	Chen
2017/0119627	A1	5/2017	Bhargava et al.
2017/0340519	A9	11/2017	Bhargava et al.
2018/0008539	A1	1/2018	Singh et al.
2018/0021284	A1	1/2018	Mégret et al.
2018/0042855	A1	2/2018	Rourke et al.
2018/0263936	A1	9/2018	Allphin et al.
2018/0318222	A1	11/2018	Allphin et al.
2019/0183806	A1	6/2019	Guillard
2019/0183836	A1	6/2019	Mégret et al.
2019/0269640	A1	9/2019	Mégret et al.
2019/0269641	A1	9/2019	Mégret et al.
2019/0274990	A1	9/2019	Mégret et al.
2019/0282532	A1	9/2019	Mégret et al.
2020/0113840	A1	4/2020	Allphin et al.
2020/0197347	A1	6/2020	Mégret et al.
2020/0276142	A1	9/2020	Grassot et al.
2020/0330393	A1	10/2020	Walsh et al.
2020/0360293	A1	11/2020	Guillard
2020/0360319	A1	11/2020	Grassot et al.
2020/0368187	A1	11/2020	Grassot et al.
2021/0186907	A1	6/2021	Skobieranda

FOREIGN PATENT DOCUMENTS

CN	102905688	A	1/2013
CN	102958930	A	3/2013
CN	103209966	A	7/2013
CN	103209967	A	7/2013
EP	0203768	A2	12/1986
EP	0235408	A1	9/1987
EP	0344704	A1	6/1989
EP	0635265	A1	7/1994
EP	0616804	A1	9/1994
EP	0635265	A1	1/1995
EP	0709087	B1	12/1999
EP	1140061	A2	10/2001
EP	1140061	B1	10/2001
EP	1316309	A1	6/2003
EP	2760911	B1	11/2017
EP	1434572	B1	12/2017
GB	922029		3/1963
GB	2295390	A	5/1996
JP	57-042651		3/1982
JP	62-12715	A	1/1987
JP	04-049212		2/1992
JP	05-508422		11/1993
JP	H06-508839	A	10/1994

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

JP	7-53365	A	2/1995
JP	H8-511257	A	11/1996
JP	09-104620	A	4/1997
JP	H10-505604	A	6/1998
JP	2001-513552	A	9/2001
JP	2002533388	A	10/2002
JP	2004-514732	A	5/2004
JP	2007-521231	A	8/2007
JP	2008-512386	A	4/2008
JP	2008-519847	A	6/2008
JP	2008-528571	A	7/2008
JP	2009-532331	A	9/2009
JP	2011-500865	A	1/2011
JP	2012507532	A	3/2012
RU	2210360	C1	8/2003
WO	WO 1994/028880	A1	12/1994
WO	WO 9640105	A1	12/1996
WO	WO 1999/009972	A1	3/1999
WO	WO 0038672	A2	7/2000
WO	WO 2002/045684	A2	6/2002
WO	WO 2005/016318	A1	2/2005
WO	WO 2005/099671	A2	10/2005
WO	WO 2006/029155	A2	3/2006
WO	WO 2006/053186	A2	5/2006
WO	WO 2006/080029	A1	8/2006
WO	WO 2007/053698	A2	5/2007
WO	WO 2007/103200	A2	9/2007
WO	WO 2008/086804	A2	7/2008
WO	WO 2009/056550	A2	5/2009
WO	WO 2010/055260	A1	5/2010
WO	WO 2010053691	A1	5/2010
WO	WO 2011/119839	A1	9/2011
WO	WO 2011/127252	A2	10/2011
WO	WO 2011/135461	A2	11/2011
WO	WO 2011/140310	A2	11/2011
WO	WO 2011139271	A1	11/2011
WO	WO 2012/028688	A1	3/2012
WO	WO 2012/107652	A1	8/2012
WO	WO 2014/078014	A2	5/2014
WO	WO 2014/093791	A1 *	6/2014
WO	WO 2015/120006	A1	8/2015
WO	WO 2015/120110	A2	8/2015
WO	WO 2015/166473	A1	11/2015
WO	WO 2016/087952	A1	6/2016
WO	WO 2016/178132	A1	10/2016
WO	WO 2017/147375	A1	8/2017
WO	WO 2017/182851	A1	10/2017
WO	WO 2018/015563	A1	1/2018
WO	WO 2019/123269	A1	6/2019
WO	WO 2020/178695	A1	9/2020

OTHER PUBLICATIONS

21 C.F.R. 184, Food and Drug Administration, HHS, (1998), pp. 441-535.

Activase, Physicians Desk Reference (50th ed.), (1996), pp. 312,1058-1061.

Akifuddin et al. "Preparation, characterization and in-vitro evaluation of microcapsules for controlled release of Diltiazem hydrochloride by Ionotropic gelation technique." Journal of Applied Pharmaceutical Science (2013); 3.4: 35-42.

Alshaikh et al., "Sodium Oxybate for Narcolepsy with Cataplexy: Systematic Review and Meta-Analysis," Journal of Clinical Sleep Medicine, 2012, vol. 8, No. 4, 451-458.

Anand et al. "Ion-exchange resins: carrying drug delivery forward." Drug Discovery Today (2001); 6.17: 905-914.

Baldrick, P., "Pharmaceutical Excipient Development: The Need for Preclinical Guidance," Regul. Toxicol. Pharmacol. Oct. 2000 32(2):210-218.

Bodmeier, R., "Tableting of coated pellets," European Journal of Pharmaceutics and Biopharmaceutics, (1997) 43(1), 1-8.

Borgen et al., "The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects." J Clin Pharmacol. (2003); 43(1): 59-65.

Borgen, L., et al. "Xyrem® (sodium oxybate): A Study of Dose Proportionality in Healthy Human Subjects." J. Clin. Pharmacol. (2000); 40:1053.

Broughton, et al. "Effects of Nocturnal Gamma-Hydroxybutyrate on Spell/Waking Patterns in Narcolepsy-Cataplexy." Can J. Neural Sci (1980); 7 (1): 23-31.

Broughton, et al. "Gamma-Hydroxy-Butyrate in the Treatment of Narcolepsy: a Preliminary Report." (1976) Narcolepsy, Ny, N.Y., Spectrum Publications, Inc. 659-668.

Caballero et al. "Characterization of alginate beads loaded with ibuprofen lysine salt and optimization of the preparation method." International Journal of Pharmaceutics (2014); 460.1: 181-188.

Chern Abstract ES302338, SciFinder®, (1964), 1 pg.

Chemical Abstracts: Seventh Collective Index, vols. 56-65, (1962-1966), 4 pgs.

Davis et al. "Active chloride secretion in the normal human jejunum." J Clin Invest. (1980); 66(6): 1326-1333.

Frucht, et al. "A pilot Tolerability and Efficacy Trial of Sodium Oxybate in Ethanol-Responsive Movement Disorders." Movement Disorders (2005); 20 (10): 1330-1337.

Gallimberti et al., "Clinical efficacy of gamma-hydroxybutyric acid in treatment of opiate withdrawal," EurArch Psychiatry Clin Neurosci. 1994;244(3):113-114.

Gallimberti et al., "Gamma-Hydroxybutyric Acid for Treatment of Opiate Withdrawal Syndrome," Neuropsychopharmacology, 1993, vol. 9, No. 1, pp. 77-81.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2019/062237, dated Mar. 31, 2020, 11 pages.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2020/066561, dated Apr. 13, 2021, 12 pages.

Jazz Pharmaceuticals, "Jazz Pharmaceuticals Announces Positive Top-line Results from Phase 3 Study of JZP-258 in Adult Narcolepsy Patients with Cataplexy and Excessive Daytime Sleepiness," Mar. 26, 2019, 2 pages, retrieved from https://investor.jazzpharma.com/node/16206/pdf.

Keating, GM, "Sodium Oxybate: A Review of Its Use in Alcohol Withdrawal Syndrome and in the Maintenance of Abstinence in Alcohol Dependence," Clinical Drug Investigation (2014) 34, 63-80.

Khediri et al., "Efficacy of Diosmectite (Smecta)® in the Treatment of Acute Watery Diarrhea in Adults: A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study," Hindawi Publishing Corporation, Gastroenterology Research and Practice, 2011, vol. 2011, Article ID 783196, 8 pages.

Lapierre et al., "The Effect of Gamma-Hydroxybutyrate: A Double-Blind Study of Normal Subjects," Sleep Research (1988); 17:99, 1988, 6 pages. (Abstract Only).

Lubrano, et al. "Fibromyalgia in Patients with Irritable Bowel Syndrome. An Association with the Severity of the Intestinal Disorder." Int J Colorectal Dis. (2001); 16 (4): 211-215.

Luhn, O., "Using Excipients in Powder Formulations," Pharmaceutical Technology Europe, Jan. 7, 2011, vol. 23, Issue 1, 6 pages, retrieved from https://www.pharmtech.com/view/using-excipients-powder-formulations.

Mahore et al. "Ion exchange resins: pharmaceutical applications and recent advancement." Int J Pharm Sci Rev Res (2010); 1.2: 8-13.

Mamelak, M., et al., "Treatment of Narcolepsy and Sleep Apnea with Gammahydroxybutyrate: A clinical and polysomnographic case study." Sleep (1981); 4 (1): 105-111.

Mamelak, M., et al., "Treatment of Narcolepsy with y-hydroxybutyrate. A review of Clinical and Sleep Laboratory Findings." Sleep (1986); 9 (1): 285-290.

Medicines for Children, "Oral Rehydration Salts," Leaflet information published Jul. 25, 2013, by Neonatal and Paediatric Pharmacists Group (NPPG), 6 pages, retrieved from https://www.medicinesforchildren.org.uk/oral-rehyd ration-salts.

Moldofsky et al. "A Chronobiologic Theory of Fibromyalgia." J. Muscoloskel. Pain, 1, 49 (1993).

Moldofsky, et al. "Musculoskeletal Symptoms and Non-REM Sleep Disturbance in Patients with 'Fibrositis Syndrome' and Healthy Subjects." Psychosom. Med. (1975); 37 (4): 341-351.

(56) **References Cited**

OTHER PUBLICATIONS

Morrison, Robert Thornton, et al., Organic Chemistry, 3rd Edition, (1973), pp. 672-677.
Ohta et al. "Development of a simple method for the preparation of a silica gel based controlled delivery system with a high drug content." European Journal of Pharmaceutical Sciences (2005); 26.1: 87-96.
Outlaw, et al. "Dyspepsia and its Overlap with Irritable Bowel Syndrome." Curr Gastroenterol Rep. (2006); 8 (4): 266-272.
Parmar et al., "Clinical Characteristics of Cataplectic Attacks in Type 1 Narcolepsy," Current Neurology and Neuroscience Reports (2020) 20:38, 9 pages.
Patil et al. "A review on ionotropic gelation method: novel approach for controlled gastroretentive gelispheres." International Journal of Pharmacy and Pharmaceutical Sciences (2012); 4.4: 27-32.
Puguan et al. "Diffusion characteristics of different molecular weight solutes in Ca-alginate gel beads." Colloids and Surfaces A: Physicochemical and Engineering Aspects (2015); 469:158-165.
Remington. The Science and Practice of Pharmacy. 20th Edition, Gennaro, Ed,. Lippincott Williams & Wilkins (2000). (See e.g. p. 861).
Remington. The Science and Practice of Pharmacy. 20th Edition, Gennaro, Ed,. Lippincott Williams & Wilkins. Chapter 45 (Oral Solid Dosage Forms) (2000) pp. 889-928.
Rohm and Haas. "Duolite AP143/1083 Pharmaceutical Grade Anion Exchange Resin." Feb. 2006, 4 pages.
Roxane Laboratories, Inc.'s Answer and Affirmative Defenses to Plaintiff's Complaint, (Jan. 4, 2013), 8 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Dec. 29, 2010), 21 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Jun. 1, 2011), 12 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Mar. 9, 2011), 13 pages.
Roxane Laboratories, Inc.'s Answer, Affirmative Defenses and Counterclaims to Plaintiff's Complaint, (Nov. 9, 2012), 18 pages.
Roxane Laboratories, Inc.'s Initial Invalidity and Noninfringement Contentions Pursuant to Local Patent Rule 3.6, (Apr. 14, 2011), 317 pages.
Rubbens et al., "Gastric and Duodenal Ethanol Concentrations after intake of Alcoholic Beverages in Postprandial Conditions," Molecular Pharmaceutics, (2017) 14(12):4202-4208.
Scharf, M. B., et al., "GHB—New Hope for Narcoleptics?" Biol Psychiatry (1989); 26 (4): 329-330.
Scrima, L., et al., "Narcolepsy." New England J. Med. (1991); 324 (4): 270-272.
Seno and Yamabe. "The Rheological Behavior of Suspensions of Ion-exchange Resin Particles." Bulletin of the Chemical Society of Japan (1966); 39.4: 776-778.
Shah et al., "In vitro Dissolution Profile Comparison—Statistics and Analysis of the Similarity Factor, f2," Pharm Research, (1998) 15(6):889-896.
Singh et al. "Ion exchange resins: drug delivery and therapeutic applications." Fabad J. Pharm. Sci (2007); 32: 91-100.
Srikanth et al., "Ion-exchange resins as controlled drug delivery carriers." Journal of Scientific Research (2010); 2.3: 597-611.
Takka and Gürel. "Evaluation of chitosan/alginate beads using experimental design: formulation and in vitro characterization." AAPS PharmSciTech (2010); 11.1: 460-466.
The Dow Chemical Company, Product Data Sheet for AMBERLITE™ IRN78 Resin. Form No. 177-02230-0311, Rev. 0, 3 pages.
Thorpy, M.J., "Recently Approved and Upcoming Treatments for Narcolepsy," CNS Drugs (2020) 34:9-27.
Transcript of a Markman Hearing, dated Apr. 26, 2012, in the case of *Jazz Pharmaceuticals, Inc.*, Plaintiff, v. *Roxane Laboratories, Inc.*, Defendant (United States District Court for the District of New Jersey, Civil 106108 ES), (Apr. 26, 2012).
Turnberg, L.A. "Abnormalities in intestinal electrolyte transport in congenital chloridorrhoea." Gut. (1971); 12(7): 544-551.
U.S. Department of Health and Human Services et al., "Dissolution Testing of Immediate Release Solid Oral Dosage Forms," Food and Drug Administration, CDER, Aug. 1997, 17 pages.
U.S. Department of Health and Human Services et al., "Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations", Food and Drug Administration, CDER, Sep. 1997, 27 pages.
Unknown author, title: definition of biotransformation; Medical dictionary; downloaded Jun. 21, 18 (Year: 2018), 3 pages.
Walden et al., "The Effect of Ethanol on the Release of Opioids 30 from Oral Sustained-Release Preparations," Drug Development and Industrial Pharmacy, 2007, 33:10,1101-1111.
Wermuth (Ed.), The Practice of Medicinal Chemistry, Academic Press, Third Edition, "Preparation of Water-Soluble Compounds Through Salt Formulation," Chapter 37, 2008, p. 758, 6 pages.
World Health Organization, "Annex 7: Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability," WHO Expert Committee on Specifications for Pharmaceutical Preparations Fortieth Report, pp. 347-390, 2006, retrieved from http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937_eng.pdf#page=359.
Zheng (Ed.), "Formulation and Analytical Development for Low-Dose Oral Drug Products," John Wiley & Sons, Inc., Hoboken, New Jersey, Table 4.1, p. 65, 2009, 3 pages.
Arena, C., et al., "Absorption of Sodium Gamma-Hydroxybutyrate and its Prodrug Gamma-Butyrolactone: Relationship Between In Vitro Transport and In Vivo Absorption," Journal of Pharmaceutical Sciences, 1980, 69(3): 356-358.
Bédard, M.A., et al., "Nocturnal Gamma-Hydroxybutyrate—Effect on Periodic Leg Movements and Sleep Organization of Narcoleptic Patients", Clin Neuropharmacol., 1989, 12(1): 29-36.
Berner, Jon E., "A Case of Sodium Oxybate Treatment of Tardive Dyskinsela and Bipolar Diorder," J. Clin Psychiatry, 2008, 69: 862.
Berthier, M, et al., "Possible Involvement of a Gamma-Hydroxybutyric Acid Receptor in Startle Disease", Acta Paediatr, 1994, 83(6): 678-680.
Broughton, Roger, et al., "The Treatment of Narcolepsy-Cataplexy with Nocturnal Gamma-Hydroxybutyrate", Le Journal Canadien des Sciences Neurologiques, 1979, 6(1): 285-289.
Erowid, "Gamma-hydroxybutyrate (GHB) Basic Synthesis Procedure," http://www.crowid.ondchemicals/ghb/ghb svnthcsis.shtm (as downloaded on Aug. 8, 2013).
European Patent Office, European Search Report for European Application Serial No. 03075658.9, dated Apr. 11, 2003, 5 pg.
Ferrara, S.D., et al., "Pharmacokinetics of Gamma-Hydroxybutyric Acid in Alcohol Dependent Patients After Single and Repeated Oral Doses", Br. J. Clin. Pharmaca., 1992, 34(3): 231-235.
Ferris, Trevor J., et al., "Synthesis, characterisation and detection of gamma-hydroxybutyrate salts", Forensic Science International, 2012, 216: 158-162.
Fides, "Solutions of 4-hydroxybutyric acid salts for injection," Chem Abstract ES302338, Laboratorio M. Cuatecases, S.A., 2011, 2 pp.
Frucht, S.J., et al., "A Single-Blind, Open-Label Trial of Sodium Oxybate for Myoclonus and Essential Tremor," Neurology, 2005, 65: 1967-1970.
Gallimberti, L., et al., "Gamma-Hydroxybutric Acid in the Treatment of Alcohol Dependence: A Double-Blind Study", Alcohol Clin. Exp. Res., 1992, 16(4): 673-676.
Gallimberti, L., et al., "Gamma-hydroxybutyric Acid for Treatment of Alcohol Withdrawal Syndrome", Clinical Pharmacology, 1989, 2(8666): 787-789.
Geekwench et al., "Title: Does anyone know why Jazz choose to make sodium oxybate?", Sep. 14, 2010; downloaded from http://www.talkaboutsleep.com/message/boards/topic/does-anybody-know-why-jazz-chose-to-make-sodium-oxybate/#sthash.no0PSCkL.dpuf on Jan. 21, 2015.
Geekwench et al., "Title: Does anyone know why Jazz choose to make sodium oxybate?", Sep. 14, 2010; downloaded from http://www.talkaboutsleep.com/message-boards/topic/does-anybody-know-why-jazz-chose-to-make-sodium-oxybate/ on Nov. 13, 2017 (30 pages).

(56) **References Cited**

OTHER PUBLICATIONS

Gerra, G., et al., "Flumazenil effects on growth hormone response to gammahydroxybutyric acid", Int Clin Psychopharmacol., 1994, 9(3): 211-215.
Gessa, G.L., "Gamma-Hydroxybutyric Acid in the Treatment of Alcohol Dependence", Clin. Neuropharm., 15 Suppl. 1, Pt. A, (1992), 303a-304a.
Gessa, Gian Luigi, et al., "Gamma-hydroxybutyric acid (GHB) for treatment of ethanol dependence", European Neuropsychopharmacology, 1993, 3(3): 224-225.
Grove-White, I.G., et al., "Critical Flicker Frequency after Small Doses of Methohexitone, Diazepam and Sodium 4-Hydroxybutyrate", Brit. J. Anaesth, 1971, 43(2): 110-112.
Grove-White, I.G., et al., "Effect of Methohexitone, Diazepam and Sodium 4-Hydroxybutyrate on Short-Term Memory", Brit. J. Anaesth., 1971, 43: 113-116.
Hasenbos, M A, "Anaesthesia for bullectomy. A technique with spontaneous ventilation and extradural blockade", Anaesthesia, 1985, 40(10): 977-980.
Hoes, M.J.A.J.M., et al., "Gamma-hydroxybutyric acid as hypnotic. Clinical and pharmacokinetic evaluation of gamma-hydroxybutyric acid as hypnotic in man", Encephale, 1980, 6(1): 93-99.
International Searching Authority, "International Search Report, dated Apr. 15, 2014, for Internaitonal Patent Application No. PCT/US2013/074954".
International Searching Authority, "Written Opinion, dated Apr. 15, 2014, for International Patent Application No. PCT/US2013/074954".
International Searching Authority, International Search Report and Written Opinion, dated Jun. 27, 2018, for International Patent Application No. PCT/EP2018/056745 (12 pages).
International Searching Authority, International Search Report for International Application Serial No. PCT/US99/30740, dated Jul. 21, 2000, 1 pg.
Jazz Pharmaceuticals, Inc., "XYREM® (sodium oxybate) oral solution Prescribing Information," XYREM® US Package Insert available at http://pp.jazzpharma.com/pi/xyrem.en.USPI.pdf (downloaded Sep. 12, 2017).
Jurkovich, Patti, Amendment filed in response to Written Opinion, International Application Serial No. PCT/US99/30740, filed Feb. 16, 2001, 9 pg.
Laborit, H., "Gamma-Hydroxybutyrate, Succinic Semialdehyde and Sleep," Laboratoire d'Eutonologie, 1973, 8: 257-274.
Ladinsky, Herbert, et al., "Mode of Action of Gamma-Butyrolactone on the Central Cholinergic System," Naunyn-Schmiedeberg's Arch. Pharmacal., 1983, 322: 42-48.
Lammers, G.J., et al., "Gammahydroxybutyrate and Narcolepsy: A Double-Blind Placebo-Controlled Study," Sleep, 1993, 16(3): 216-220.
Lapierre, O., et al., "The Effect of Gamma-Hydroxybutyrate on Nocturnal and Diurnal Sleep of Normal Subjects: Further Considerations on REM Sleep-Triggering Mechanisms," Sleep, 1990, 13(1): 24-30.
Lee, C.R., "Evidence for the Beta-Oxidation of Orally Administered 4-Hydroxybutyrate in Humans", Biochemical Medicine, 1977, 17(3): 284-291.
Lettieri, John, et al., "Improved Pharmacological Activity via Pro-Drug Modification: Comparative Pharmacokinetics of Sodium Gamm-Hydroxybutyrate and Gamma-Butyrolactone", Research Communications in Chemical Pathology and Pharmacology, 1978, 22(1): 107-118,.
Lynch, M., "Malic Acid", The Handbook of Pharmaceutical Excipients, 2nd Ed., 1994, 63 3: 285-286.
Mamelak, M., et al., "Sleep-Inducing Effects of Gammahydroxybutyrate", The Lancet, 1973, 2(7824): 328-329.
Mamelak, Mortimer, "Gammahydroxybutyrate: An Endogenous Regulator of Energy Metabolism", Neuroscience and Biobehavioral Reviews, 1989, 13(4): 187-198.
Mamelak, Morty, et al., "The Effects of Gamma-Hydroxybutyrate on Sleep", Biological Psychiatry, 1977, 12(2): 273-288.
Morrison, Robert T., et al., "Organic Chemistry", Chapter 20: "Functional Derivatives of Carboxylic Acids," 3rd Edition, 1973, pp. 658-700.
Nema, Sandeep, et al., "Excipients and Their Use in Injectable Products", PDA J. Pharm. Sci. Technol, 1997, 51(4): 166-171.
Neuman, Ariel, "GHB's Path to Legitimacy: An Administrative and Legislative History of Xyrem", paper submitted to Harvard Law School, 2004, 1-39.
Ondo, William G., et al., "Sodium Oxybate for Excessive Daytime Sleepiness in Parkinson Disease," Arch. Neural., 2008, 65(10): 1337-1340.
Palatini, P., et al., "Dose-Dependent Absorption and Elimination of Gamma-Hydroxybutyric Acid in Healthy Volunteers", Eur. J. Clin Pharmacal., 1993, 45(4): 353-356.
Roth, R. H., et al., "Gamma-Butyrolactone and Gamma-Hydroxybutyric Acid-II. The Pharmacologically Active Form", J. Neuropharmacol. 1966, 5: 421-428.
Roth, Robert H., et al., "Gamma-Butyrolactone and Gamma-Hydroxybutyric Acid-I, Distribution and Metabolism", Biochemical Pharmacology, 1966, 15: 1333-1348.
Russel, I. Jon, et al., "Sodium Oxybate Relieves Pain and Improves Function in Fibromyaligia Syndrome," Arthritis. Rheum , 2009, 60: 299-309.
Scharf et al., "Effect of Gamma-Hydroxybutyrate on Pain, Fatigue, and the Alpha Sleep Anomaly in Patients with Fibromyalgia. Preliminary Report", The Journal of Rheumatology, 25(10): 1986-1990 (1998).
Scharf, M.B., et al., "The Effects and Effectiveness of Gamma-Hydroxybutyrate in Patients with Narcolepsy", J. Clin. Psychiatry, 1985, 46(6): 222-225.
Scharf, Martin B., et al., The Effects of Sodium Oxybate on Clinical Symptoms and Sleep Patterns in Patients with Fibromyalgia, J. Rheumatol, 2003, 30(5): 1070-1074.
Scrima, et al., "Effect of High Altitude on a Patient with Obstructive Sleep Apnea", Sleep Research, Abstract, 1987, 16: 427.
Scrima, et al., "Effects of Gamma-Hydroxybutyrate (GHB) on Narcolepsy-Cataplexy Symptoms and MSLT Results in Male and Female Patients", Association of Professional Sleep Societies, Abstract, 1988, 251.
Scrima, et al., "Gamma-Hydroxybutyrate Effects on Cataplexy and Sleep Attacks in Narcoleptics", Sleep Research, Abstract, 1987, 16: 134.
Scrima, L, et al., "Efficacy of Gamma-Hydroxybutyrate Versus Placebo in Treating Narcolepsy-Cataplexy: Double-Blind Subjective Measures", Biol. Psychiatry, 1989, 26(4): 331-343.
Scrima, L. et al., "Effect of Gamma-Hydroxybutyrate on a Patient with Obstructive Sleep Apnea," Sleep Research, Abstract, 1987, 16: 137.
Scrima, Lawrence, et al., "The Effects of Gamma-Hydroxybutyrate on the Sleep of Narcolepsy Patients: A Double-Blind Study", Sleep, 1990, 13(6): 479-490.
Sériès, F., et al., "Effects of Enhancing Slow-Wave Sleep by Gamma-Hydroxybutyrate on Obstructive Sleep Apnea", Am. Rev. Respir. Dis., 1992, 145(6): 1378-1383.
Snead, O. Carter et al., "Ontogeny of Gamma-Hydroxybutyric Acid. I. Regional Concentration in Developing Rat, Money and Human Brain," Brain Res., 1981, 227(4): 579-589.
Snead, O. Carter, "Gamma-Hydroxybutyrate Model of Generalized Absence Seizures: Further Characterization and Comparison with Other Absence Models," Epilepsia, 1988, 29(4): 361-368.
Stock, Günter, et al., "Increase in Brain Dopamine after Axotomy or Treatment with Gammahydroxybutyric Acid Due to Elimination of the Nerve Impulse Flow", Naunyn-Schmiedeberg's Arch, Pharmacal., 1973, 278(4): 347-361,.
Strong, A. J., "Gamma-Hydroxybutyric Acid and Intracranial Pressure", The Lancet, 1984, 1(8389): 1304.
Suner, S., et al., "Pediatric Gamma Hydroxybutyrate Intoxication", Acad. Emerg. Med., 1997, 4(11): 1041-1045.
Tunnicliff, Godfrey, "Sites of Action of Gamma-Hydroxybutyrate (GHB)—A Neuroactive Drug with Abuse Potential", Clinical Toxicology, 1997, 35(6): 581-590.

(56) **References Cited**

OTHER PUBLICATIONS

United States District Court, "Opinion," *Jazz Pharmaceuticals, Inc.* v. *Roxane Laboratories, Inc.*, Markman Hearing, No. 10-6108 (ES), (Sep. 14, 2012), 43 pg.

United States District Court, "Order," *Jazz Pharmaceuticals, Inc.* v. *Roxane Laboratories, Inc.*, Markman Hearing, No. 10-6108 (ES), (Sep. 14, 2012), 1 pg.

United States Pharmacopeia (USP), Pharmaceutic Ingredients, 23/NF18, 1995, p. 2205.

Van Den Bogert, et al., "Placentatransfer of 4-Hydroxybutyric Acid in Man", Anaesthesiology and Intensive Care Medicine, 1978, 110: 55-64.

Vickers, M.D., "Gammahydroxybutyric Acid", Int. Anesth. Clinic, 1969, 7(1): 75-89.

Vogel et al., 2018, "Toxicologic/transport properties of NCS-382, a γ-hydroxybutyrate (GHB) receptor ligand, in neuronal and epithelial cells: Therapeutic implications for SSADH deficiency, a GABA metabolic disorder," Toxicol In Vitro, 46:203-212 (Epub 2017),.

Yamada, Y., et al., "Effect of Butyrolactone and Gamma-Hydroxybutyrate on the EEG and Sleep Cycle in Man", Electroenceph. clin. Neurophysiol., 1967, 22: 558-562.

Chen et al., "Pharmacokinetics, relative bioavailability and food effect of JZP-258 and sodium oxybate: results of two phase 1, open-label, randomised crossover studies in healthy volunteers," Sleep Medicine, Abstracts, 2019, vol. 64, pp. S65-S66.

International Search Report and Written Opinion of the International Searching Authority for International Application No. PCT/US2021/019024, dated Jun. 2, 2021, 10 pages.

Jha, M.K, "Modified release formulations to achieve the quality target product profile (QTPP)," IJPSR, 2012; vol. 3(8): 2376-2386.

Rujivipat et al., "Improved drug delivery to the lower intestinal tract with tablets compression-coated with enteric/nonenteric polymer powder blends," European Journal of Pharmaceutics and Biopharmaceutics (2010) 76: 486-492.

Non-Final Office Action dated Aug. 25, 2021, for U.S. Appl. No. 17/222,579, 14 pages.

* cited by examiner

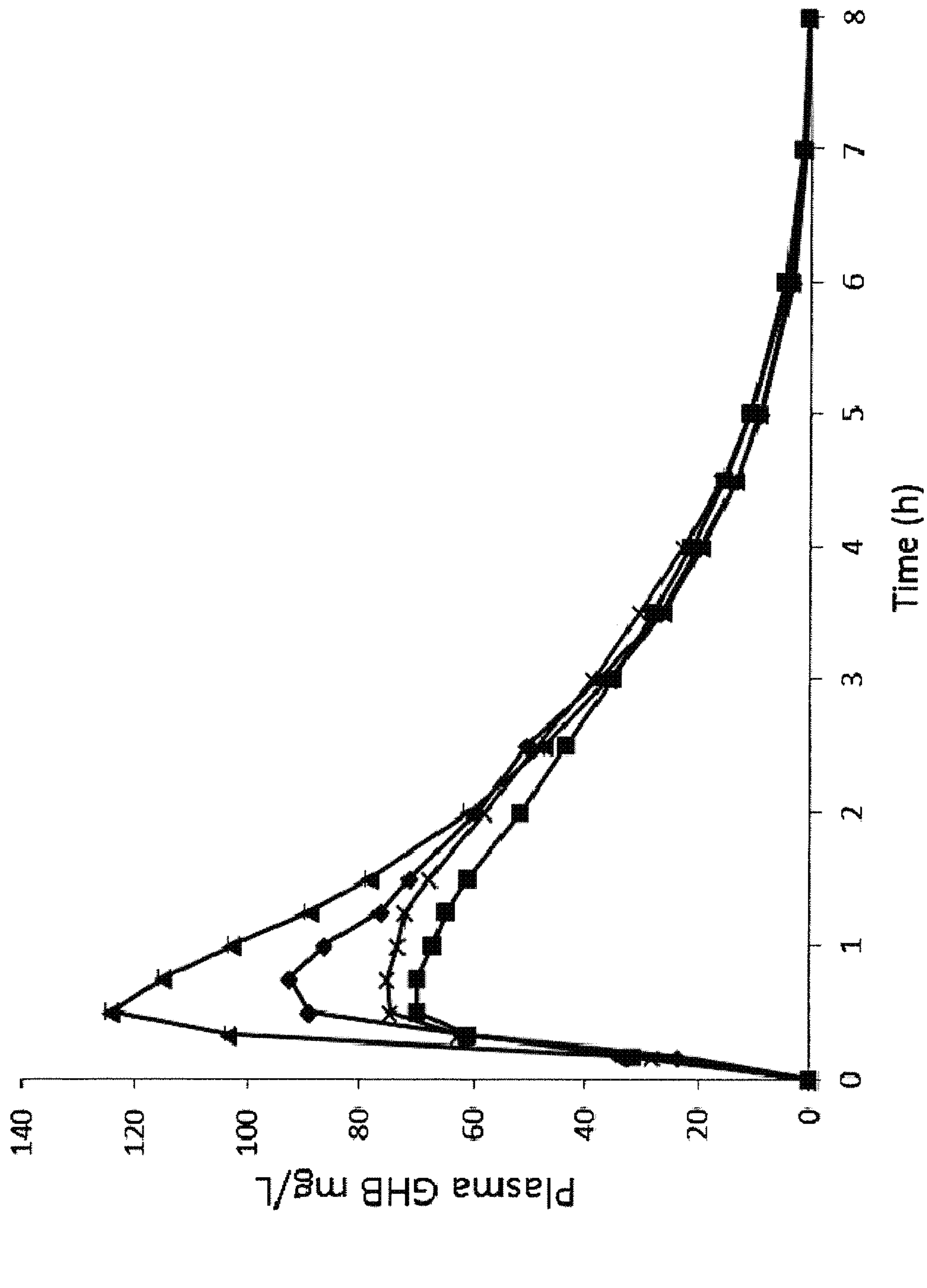


FIG. 1

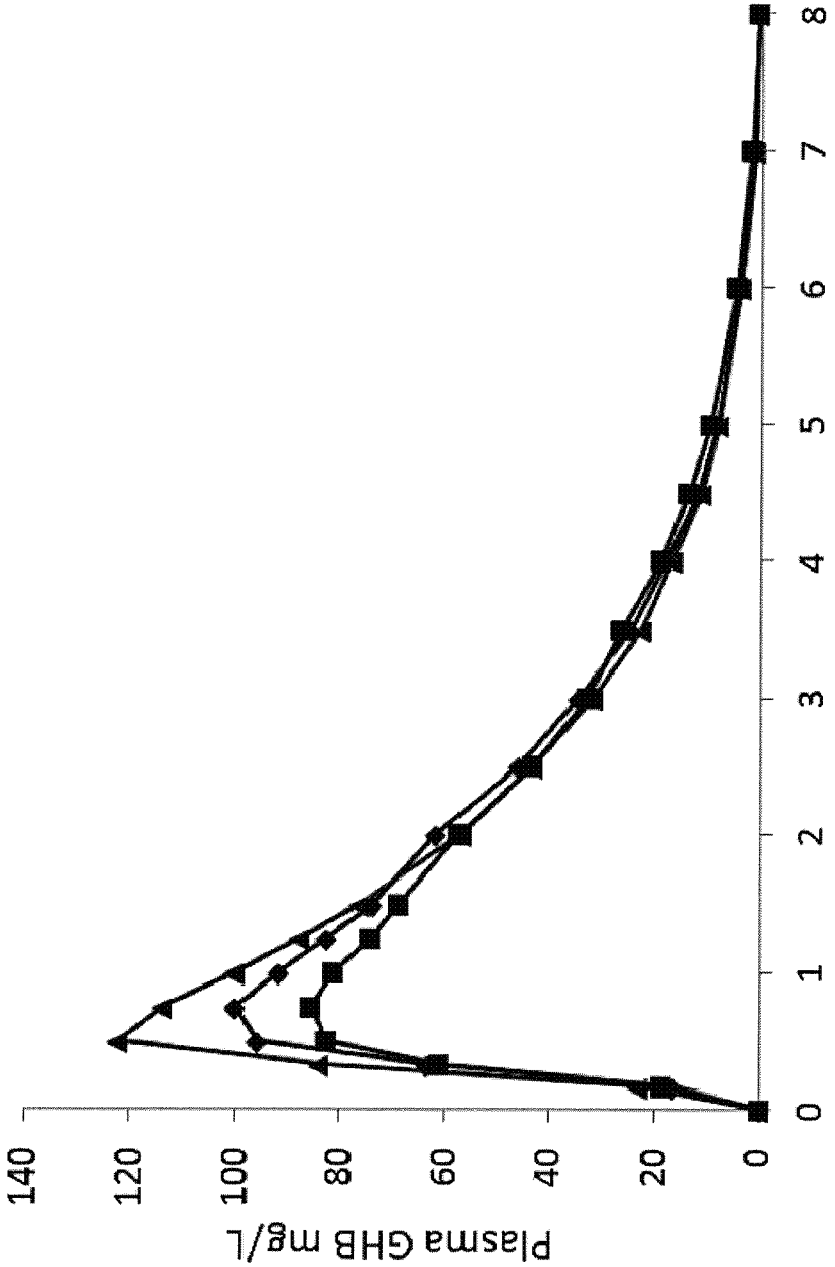


FIG. 2

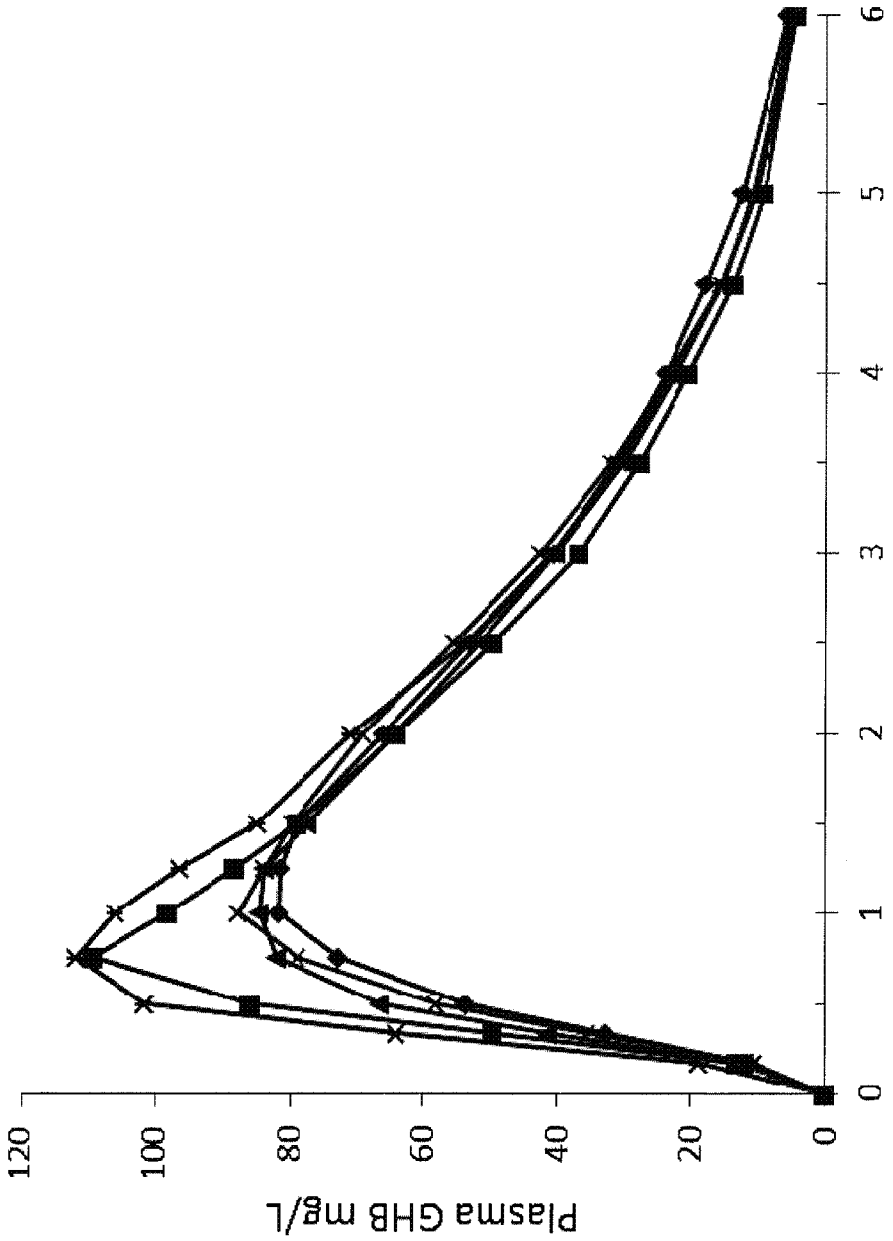


FIG. 3

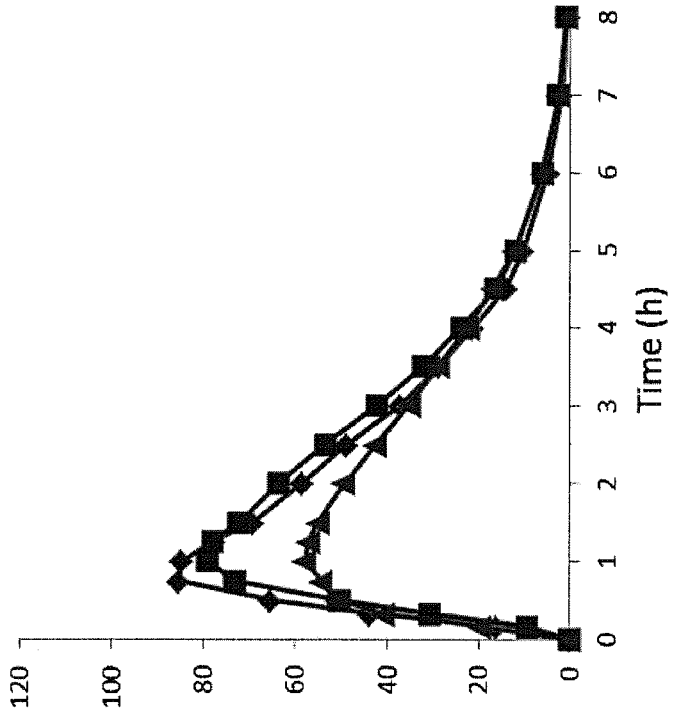

120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Time (h)

FIG. 4B

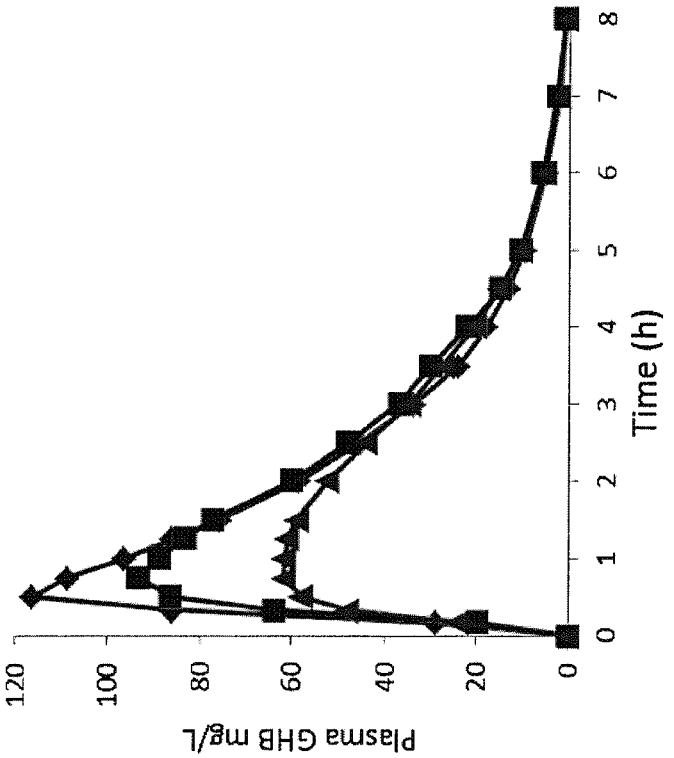

Plasma GHB mg/L
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Time (h)

FIG. 4A

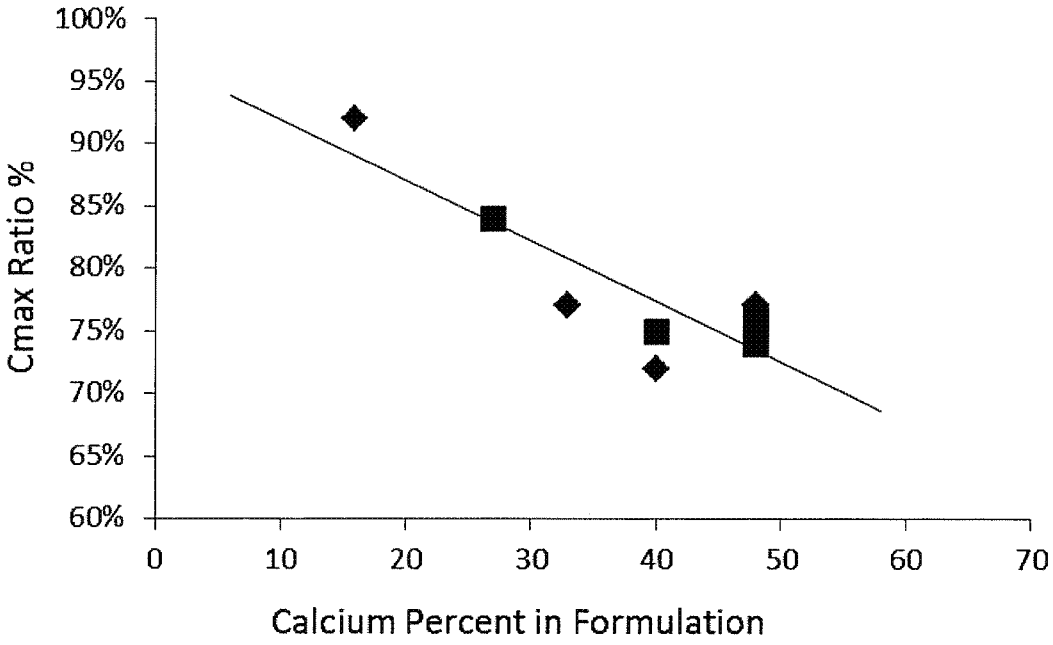
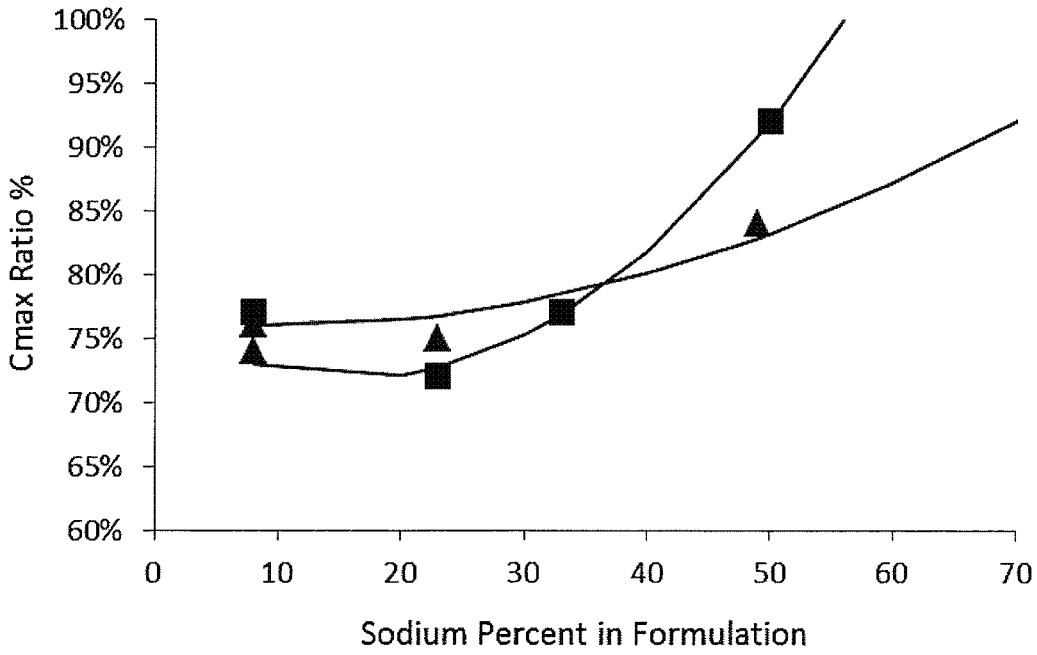

100%
95%
90%
85%
80%
75%
70%
65%
60%
Cmax Ratio %
0
10
20
30
40
50
60
70
Calcium Percent in Formulation

FIG. 5A


100%
95%
90%
85%
80%
75%
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65%
60%
Cmax Ratio %
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20
30
40
50
60
70
Sodium Percent in Formulation

FIG. 5B

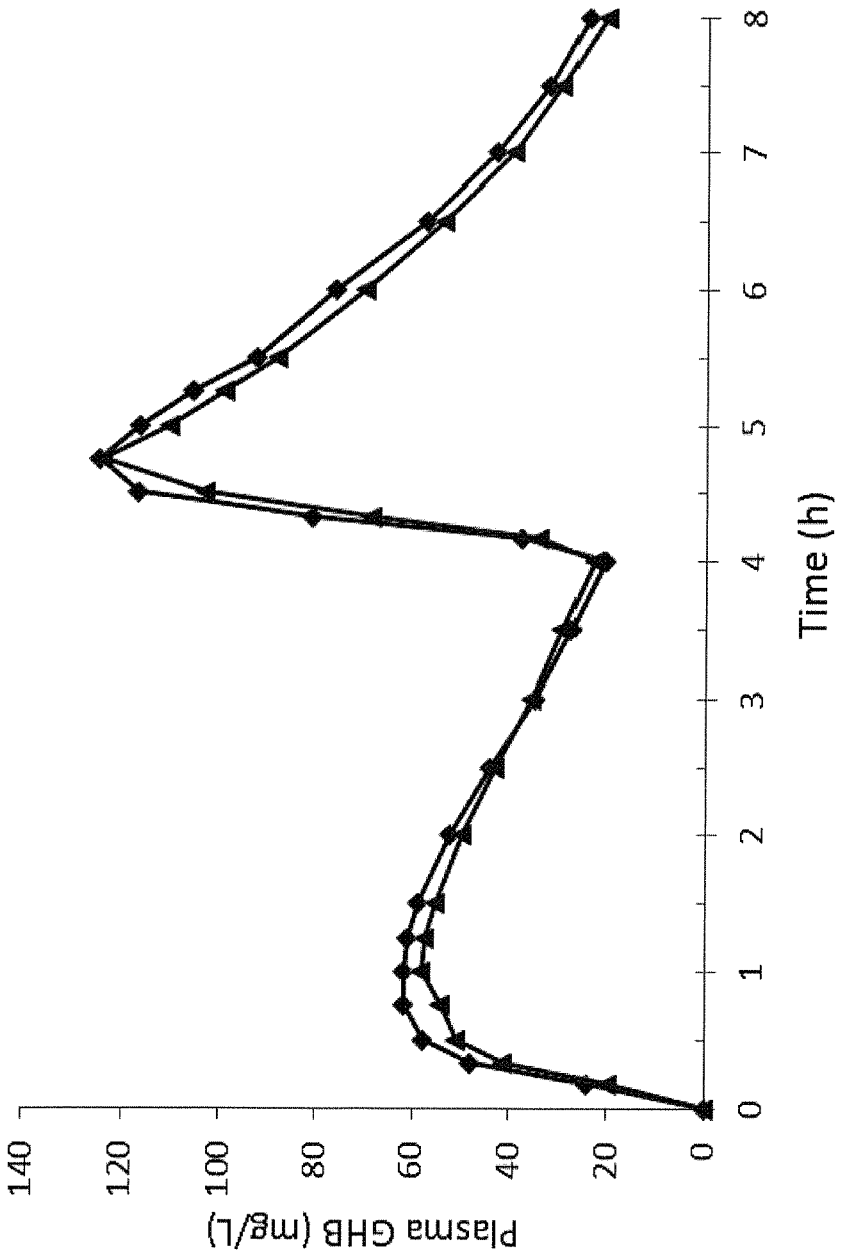


FIG. 6

GAMMA-HYDROXYBUTYRATE COMPOSITIONS AND THEIR USE FOR THE TREATMENT OF DISORDERS

1. CROSS REFERENCE

This application is a continuation of U.S. patent application Ser. No. 16/575,213, filed Sep. 18, 2019, which is a continuation of U.S. patent application Ser. No. 15/709,262, filed Sep. 19, 2017, now abandoned, which claims the benefit of U.S. Provisional Patent Application No. 62/473,232, filed Mar. 17, 2017, the content of each of which is incorporated herein by reference in its entirety.

2. FIELD OF THE INVENTION

Provided herein are pharmaceutical compositions and formulations comprising salts of gamma-hydroxybutyrate (GHB). In one embodiment, the salts encompass more than one type of cation. Also provided herein are methods of making the pharmaceutical compositions and formulations, and methods of the treatment of disorders including fibromyalgia and sleep disorders. Also described herein is that such pharmaceutical compositions and formulations are for treating diseases or disorders including fibromyalgia and sleep disorders. Such sleep disorders include apnea, sleep time disturbances, narcolepsy, cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

3. BACKGROUND OF THE INVENTION

Sodium oxybate (Na.GHB), commercially sold as Xyrem® (Jazz Pharmaceuticals), is approved for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Na.GHB has also been reported to be effective for relieving pain and improving function in patients with fibromyalgia syndrome (See Scharf et al., 2003, *J. Rheumatol.* 30: 1070; Russell et al., 2009, *Arthritis. Rheum.* 60: 299), and in alleviating excessive daytime sleepiness and fatigue in patients with Parkinson's disease, improving myoclonus and essential tremor, and reducing tardive dyskinesia and bipolar disorder (See Ondo et al., 2008, *Arch. Neural.* 65: 1337; Frucht et al., 2005, *Neurology* 65: 1967; Berner, 2008, *J. Clin. Psychiatry* 69: 862).

Xyrem®, for use with patients with narcolepsy, is a chronically used product which requires high levels of the drug. The amount of sodium intake from the drug significantly increases the daily sodium intake for patients, which is undesirable for patients with hypertension, heart disease, renal disease or at risk of stroke.

Since Xyrem® is administered to a broad population, there is a need for GHB formulations that minimize the undesirable side effects of the sodium, particularly in patients with hypertension, heart disease, renal disease or at risk of stroke, yet provide additional health benefits from the presence of the other salts. It is desirable that such modified formulations provide good solubility, stability and purity in order to provide safe, effective and consistent doses to patients, and also display acceptable pharmacodynamic and pharmacokinetic properties. See U.S. Pat. Nos. 8,591,922; 8,901,173; and 9,132,107; which are incorporated by reference in their entireties.

4. SUMMARY OF THE INVENTION

Provided herein are pharmaceutical compositions and formulations comprising salts of gamma-hydroxybutyrate

("GHB") which are useful in the treatment of conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, excessive daytime sleepiness (EDS) cataplexy, sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

One embodiment, as provided herein, is a GHB formulation with a reduction in sodium content. Another embodiment, as provided herein, is a GHB formulation with a reduced sodium content and which is bioequivalent to Xyrem®. In certain embodiments, the reduction in sodium content involves use of other cations such as potassium, calcium, magnesium, and others.

For convenience in comparing various salt compositions at the same oxybate or GHB molar dose, compositions expressed as percentages in this application refer to molar equivalent percentage (% molar equivalents) of each salt of oxybate or GHB. This is usually close to, but not the same as, a composition that would be expressed as wt/wt %. As used herein, the terms "oxybate" and "GHB" are used interchangeably.

Accordingly, in one aspect, provided herein are pharmaceutical compositions and formulations comprising salts of GHB. In one embodiment, the formulation is a pharmaceutical composition of GHB comprising a mixture of two or more salts of GHB, wherein the mixture comprises at least 50% of a sodium salt of gamma-hydroxybutyrate (Na.GHB), and wherein the mixture further comprises one or more of a potassium salt of gamma-hydroxybutyrate (K.GHB) and a calcium salt of gamma-hydroxybutyrate (Ca.$(GHB)_2$). In certain embodiments, the Na.GHB salt is present in the mixture in about 50%, and up to 55%, 60%, 70% or 80%. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of a magnesium salt of gamma-hydroxybutyrate (Mg.$(GHB)_2$).

In another embodiment the pharmaceutical composition is given to the patient in an aqueous solution with a volume of between 25 and 100 mL, 25 and 75 mL, or 55 and 65 mL.

In another embodiment, the pharmaceutical composition, when administered to a patient, is bioequivalent to the average maximum GHB plasma concentration (Cmax) and the average maximum GHB plasma area under the curve (AUC) of the Cmax of Na.GHB within 80% to 125%.

In another embodiment, the pharmaceutical composition comprises a mixture of three salts of GHB, wherein the mixture comprises at least 50% of Na.GHB, and further comprises K.GHB and Ca.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises between 50 and 60% of Na.GHB, and further comprises between 20 and 40% K.GHB, and between 10 and 20% Ca.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises about 50% of Na.GHB, 34% K.GHB, and 16% Ca.$(GHB)_2$ for each GHB salt.

In another embodiment, the pharmaceutical compositions and/or formulations disclosed herein can be used to treat a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure.

In another embodiment, the pharmaceutical compositions disclosed herein comprise less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture

of two or more GHB salts, the mixture comprising between 40% to 50% Na.GHB and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical compositions disclosed herein do not comprise a substantial amount Ca.$(GHB)_2$) or Mg.$(GHB)_2$.

In another embodiment, the pharmaceutical composition comprises about 8% Na.GHB, 23% K.GHB, 48% Ca.$(GHB)_2$ and 21% Mg.$(GHB)_2$. In certain embodiments, this pharmaceutical composition can be used to treat the diseases or conditions listed above.

In another embodiment, the pharmaceutical compositions and/or formulations disclosed herein, when administered to a patient, have a lower average maximum GHB plasma concentration (Cmax) than the Cmax of Na.GHB.

Xyrem®, as disclosed herein, is a commercially sold product comprised of 100% sodium oxybate (Na.GHB), and is prescribed for twice nightly use for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Accordingly, in another aspect, provided herein is a first dose of a first pharmaceutical composition and/or formulation having a Na.GHB of less than 50% and a second dose of a second pharmaceutical composition and/or formulation having a Na.GHB above 50%. Another embodiment has the doses in reverse order and a further embodiment uses similar doses of either formulation. In certain embodiments, the first dose can be administered within 4 hours of eating and produces a GHB Cmax lower than the Cmax of Na.GHB, but may have less of a food effect.

In another aspect, the pharmaceutical compositions and formulations provided herein can be used to treat a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In one embodiment, the formulations and pharmaceutical compositions provided herein can be used to treat conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

The pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g. Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In certain embodiment, the formulations and pharmaceutical compositions disclosed herein are used in a method of treating conditions responsive to GHB, for example, fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In another aspect, provided herein are methods of treating a disease or condition in a patient that is suitable for treatment with GHB, comprising administering to the patient the pharmaceutical compositions and formulations disclosed herein. In certain embodiments, the disease or condition is selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder (e.g., Parkinson's Disease and depression), an endocrine disturbance, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In certain embodiments, the disease or condition is elected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In another embodiment, methods of treatment disclosed herein comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering to a patient having a disease or condition that is suitable for treatment with GHB the first dose; and (iv) orally administering to the patient the second dose within 2.5 to 4 hours following the first dose.

The pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition in a patient that is suitable for treatment with GHB, comprising administering to the patient the pharmaceutical compositions and formulations disclosed herein.

In certain embodiments, the pharmaceutical compositions and formulations disclosed herein is for use in a method of treating a disease or condition in a patient further comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering to a patient having a disease or condition that is suitable for treatment with GHB the first dose; and (iv) orally administering to the patient the second dose within 2.5 to 4 hours following the first dose.

In other aspects, provided herein are methods of making the pharmaceutical compositions disclosed herein.

5. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows the plasma GHB concentration vs time for Formulation "O" (8% Na.GHB, 23% K.GHB, 48% Ca.(GHB), and 21% Mg.$(GHB)_2$) compared to Xyrem® ("X") given in either the fed or fasted state (✱, Xyrem® fasted; ✚, Formulation "O" fasted; ✳ Xyrem® fed; ■, Formulation "O" fed). The objective was to characterize bioequivalence of Formulation "O" to Xyrem®.

FIG. **2** shows the plasma GHB concentration vs time for blends of Formulation "O" and Xyrem® ("X") in proportions of 100% Xyrem®, 44% Xyrem®, and 17% Xyrem®, respectively (✱, fasted 4.5 g "X"; ✚, fasted 2.5 g "O"+2.0 g "X"; ■, fasted 3.75 g "O"+0.75 g "X"). The

objective was to determine how much sodium (or Xyrem®) would be required to achieve bioequivalence in the fasted state.

FIG. **3** shows the plasma GHB concentration vs time for various mixed oxybate salt formulations compared to Xyrem® in the fasted state where both are given at a lower volume of administration of 60 mL (, Xyrem® (100% Na); , Formulation 507D (50% Na, 34% K, 16% Ca, 0% Mg); , 507C (33% Na, 0% K, 48% Ca, 19% Mg); , 507A (33% Na, 34% K, 33% Ca, 0% Mg); , 507G (23% Na, 19% K, 40% Ca, 18% Mg)).

FIG. **4**A-**4**B compare Xyrem® and Formulation "O" when given fasted with 60 mL or 240 mL water or when given fed with 60 mL water. FIG. **4**A. (Left) Plasma GHB concentration when Xyrem® was given (fasted) with 60 mL or 240 mL water or when Xyrem® was given (fed) with 60 mL water (, fasted 240 mL; , fasted 60 mL; , fed 60 mL). FIG. **4**B (Right) Plasma GHB concentration when Formulation "O" was given (fasted) with 60 mL or 240 mL water or when Formulation "O" was given (fed) with 60 mL water (, fasted 240 mL; , fasted 60 mL; , fed 60 mL).

FIG. **5**A-**5**B show the relationship between Cmax ratio (to Xyrem®) and calcium content or sodium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume or 60 mL aqueous volume. FIG. **5**A. (Top) Relationship between Cmax ratio (to Xyrem®) and calcium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume (, Cmax, 60 mL; , Cmax, 240 mL). FIG. **5**B (Bottom) Relationship between Cmax ratio (to Xyrem®) and sodium content of the example formulations subjected to fasted-state PK evaluations when administered in either 240 mL aqueous volume (, Cmax, 60 mL; , Cmax, 240 mL).

FIG. **6** is a graph showing the expected behavior of taking separate formulations as part of an equally divided dose given 4 h apart (, 1st dose Xyrem® fed, 2nd dose Xyrem® fasted; , 1st dose Formulation "O" fed, 2nd dose Formulation 507D fasted). Formulation "O" is given initially and then formulation "507D" is given 4 h later. This is compared to Xyrem® given both times.

6. DETAILED DESCRIPTION OF THE INVENTION

Gamma-hydroxybutyrate (GHB), also known as "oxybate," is an endogenous compound with hypnotic properties that is found in human body tissues, such as the mammalian brain. In the brain, the highest GHB concentration is found in the hypothalamus and basal ganglia and GHB is postulated to function as a neurotransmitter (See Snead and Morley, 1981, *Brain Res.* 227(4): 579-89). The neuropharmacologic effects of GHB include increases in brain acetylcholine, increases in brain dopamine, inhibition of GABA-ketoglutarate transaminase and depression of glucose utilization but not oxygen consumption in the brain. GHB treatment substantially reduces the signs and symptoms of narcolepsy, i.e., daytime sleepiness, cataplexy, sleep paralysis, and hypnagogic hallucinations. In addition, GHB increases total sleep time and REM sleep, and it decreases REM latency, reduces sleep apnea, and improves general anesthesia (see, e.g., U.S. Pat. Nos. 6,472,431; 6,780,889; 7,262,219; 7,851,506; 8,263,650; 8,324,275; and 8,772,302 each of which is incorporated herein by reference in its entirety).

Xyrem® is a commercially sold product comprised of 100% sodium oxybate (Na.GHB) and is approved for the treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. Na.GHB has also been reported to be effective for relieving pain and improving function in patients with fibromyalgia syndrome, and in alleviating excessive daytime sleepiness and fatigue in patients with Parkinson's disease, improving myoclonus and essential tremor, and reducing tardive dyskinesia and bipolar disorder. See the references that are incorporated at the end of U.S. Pat. No. 6,472,431. Further, despite a general record of safety when used as prescribed, there are risks of abuse and misuse of Xyrem® which can cause serious medical problems, including seizures, loss of consciousness, coma, and death (see, e.g., FDA product label dated Nov. 13, 2006 for NDA no. 021196, which is incorporated by reference in its entirety).

Xyrem® for use with patients with narcolepsy, is a chronically used product which requires high levels of the drug. The amount of sodium intake from the drug significantly increases the daily sodium intake for patients, which is undesirable for patients with hypertension, heart disease, renal disease or at risk of stroke. Thus, there is a need for GHB formulations with lower sodium, such as those provided herein, particularly for patients with hypertension, heart disease, renal disease or at risk of stroke, yet provide additional health benefits from the presence of the other salts.

However, the therapeutic dose of 71.4 mEq/day (9 g sodium oxybate) is sufficiently high that shifting from sodium to another cation can push limits on acceptable daily intake of other cations and potentially cause other problems for certain patients. For example, potassium has poor tolerability in solution at high doses given on an empty stomach and can also be problematic for patients with kidney impairment. Therefore, formulations which reduce or eliminate sodium without exceeding levels of concern for other cations are particularly desirable.

Xyrem® is provided as an oral solution consisting of 500 mg/mL sodium oxybate (Na.GHB) that is pH adjusted with malic acid. Xyrem® is rapidly and well absorbed when given on an empty stomach. The absolute bioavailability for 2.25 g and 4.45 g sodium oxybate doses, relative to IV administration, is 88%. See the Xyrem® Product Insert. As a result, sodium oxybate is generally considered to be a high solubility, high permeability drug. (See Yu et al., *Pharm. Res.* 19 (7) 921-925). As such, for alternative formulations of GHB, such as those comprising cations other than sodium, but having comparable solubility, bioequivalence might be expected and a pharmacokinetic evaluation waived. See 21 CFR Part 320.22 Subpart B paragraph b(3).

However, as disclosed herein, despite the apparently rapid absorption of sodium oxybate, its presentation as an aqueous solution, and the absence of any other ingredients that would be expected to modify absorption behavior, formulations having the same GHB concentration do not display pharmacokinetics equivalent to Xyrem®. Furthermore, as also disclosed herein, the pharmacokinetic behavior of such formulations appears to depend on the amount of sodium and/or other cations present, as well as the amount of water in the formulation. Accordingly, one object of the present disclosure is to provide alternative formulations of GHB which are bioequivalent to Xyrem®. Provided herein are such alternative formulations which surprisingly display the desired bioequivalence.

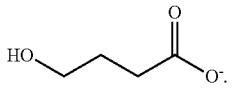
The following patents and applications referred to throughout the application are hereby incorporated by ref-

erence in their entireties for all purposes, including the following: U.S. Pat. Nos. 6,472,431; 7,895,059; 8,461,197; 8,591,922; 8,759,394; 8,771,735; 8,772,306; 8,778,301 8,778,398; 8,952,029; and 9,050,302; and U.S. Publication No. 2012/0076865.

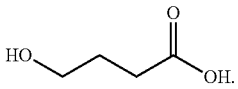
Objects, features and advantages of the methods and compositions described herein will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

6.1 Definitions

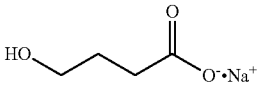
As used herein, the term "gamma-hydroxybutyrate" (GHB) or "oxybate" refers to the negatively charged or anionic form (conjugate base) of gamma-hydroxybutyric acid. Without being limited by theory, GHB is believed to have the following structure:



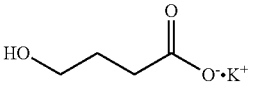
As used herein, the term "gamma-hydroxybutyric acid" refers to the protonated form (conjugate acid) of gamma-hydroxybutyrate. Without being limited by theory, gamma-hydroxybutyric acid is believed to have the following structure:



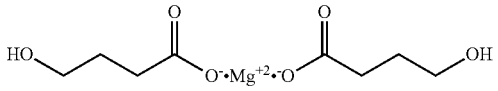
As used herein, the terms "sodium gamma-hydroxybutyrate" (Na.GHB) or "sodium oxybate" (Na.oxybate) refers to the sodium salt form of gamma-hydroxybutyric acid having the molecular weight of 126.09. Without being limited by any theory, Na.GHB is believed to have the following structure:



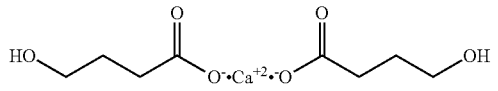
As used herein, the term "potassium gamma-hydroxybutyrate" (K.GHB) or "potassium oxybate" (K.oxybate) refers to the potassium salt form of gamma-hydroxybutyric acid having the molecular weight of 142.19. Without being limited by any theory, K.GHB is believed to have the following structure:



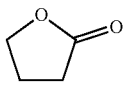
As used herein, the term "magnesium gamma-hydroxybutyrate" ($Mg.(GHB)_2$) or "magnesium oxybate" (Mg.oxybate) refers to the magnesium salt form of gamma-hydroxybutyric acid having the molecular weight of 230.50. Without being limited by theory, $Mg.(GHB)_2$ is believed to have the following structure:



As used herein, the term "calcium gamma-hydroxybutyrate" ($Ca.(GHB)_2$) or "calcium oxybate" (Ca.oxybate) refers to the calcium salt form of gamma-hydroxybutyric acid having the molecular weight of 246.27. Without being limited by theory, $Ca.(GHB)_2$ is believed to have the following structure:



As used herein, the term "gamma-butyrolactone" (GBL) refers to a colorless oily liquid. Without being limited by theory, GBL is believed to have the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

The terms "treat," "treating" or "treatment," as used herein, refer to a method of alleviating or abrogating a disease and/or its attendant symptoms.

As used herein, the term "about" or "approximately" means an acceptable error for a particular value as determined by those skilled in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term "about" or "approximately" means within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value.

The term "substantial amount" shall mean over 1%.

By "pharmaceutically acceptable" it is meant the active ingredient, cation, salt, diluent, excipient or carrier must be compatible with the other ingredients of the formulation and not unduly deleterious, for example, that the active ingredient, cation, salt, diluent, excipient or carrier does not produce an adverse, allergic or other untoward reaction, when administered to an animal, or a human, as appropriate.

The term "salt" or "salts," as used herein, refers to a compound formed by the interaction of an acid and a base, the hydrogen atoms of the acid being replaced by the positive ion or cation of the base. Pharmaceutically acceptable salts, include inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as malic, acetic, oxalic, tartaric, mandelic, and the like. Salts formed can also be derived from inorganic bases such as, for example, sodium, potassium, silicates, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. In certain preferred embodiments, the salt is formed from an

inorganic base that is a metal, for example, an alkali metal, such as lithium, potassium, sodium, or the like, an alkaline earth metal, such as magnesium, calcium, barium, or the like, or aluminum or zinc. Other salts may comprise ammonium. Alkali metals, such as lithium, potassium, sodium, and the like, may be used, preferably with an acid to form a pH adjusting agent. Examples of pharmaceutically acceptable base addition salts include those derived from inorganic bases like sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, or ammonium hydroxide, and the like (See, e.g., Berge et al., 1977, *J. Pharm. Sci.* 66: 1).

As used herein, the terms "salt of GHB" or "salts of GHB," as used herein, refer to a compound formed by the interaction of gamma-hydroxybutyric acid (the conjugate acid of GHB) with a base, for example, NaOH, KOH, $Mg(OH)_2$, and $Ca(OH)_2$, and the like, the hydrogen atoms of the acid being replaced by the positive ion or cation of the base. Such salts may include, for example, Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$, and the like. It will be understood by those skilled in the art that such salts may be in solid form, or such salts may be in partially or fully solvated form, for example, as when dissolved in an aqueous medium. It will be further understood by those skilled in the art, that, depending on the solubility of the salt in the aqueous medium, that the salt may be present in the aqueous medium as solvated cation(s) and anion(s), or as a precipitated solid, as illustrated below for the solubility equilibrium of $Ca.(GHB)_2$:

$$Ca{\bullet}(GHB)_{2(s)} \overset{H_2O}{\rightleftharpoons} Ca^{+2}_{(aq)} + 2\,(GHB)^{-}_{(aq)}$$

The terms "mixture of salts" or "salt mixture," as used herein, refers to salts of GHB where two or more different cations are present in combination with each other in a composition. Such mixtures of salts may include, for example, two or more salts selected from the group consisting of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$.

Xyrem® contains 500 mg/mL Na.GHB. When referring to a mixture of GHB salts with different cations, the concentration in mg/mL will vary between formulations and/or pharmaceutical compositions of the same GHB strength. As used herein, a GHB concentration of 409 mg/mL is equivalent to the GHB content in 500 mg/mL of Na.GHB.

The term "wt/wt %," are used herein, refers to the normalized weight percent of a particular salt in a salt mixture. A sample calculation of wt/wt % is provided in Example 1 of the present disclosure.

The term "wt/wt % ratio," as used herein, refers to the ratio of wt/wt % values in a mixture of salt. For example, where the salts Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$ are present in a wt/wt %'s of 8%, 32%, 20% and 40%, respectively, the wt/wt % ratio of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$ in the mixture is 8%:32%:20%:40%.

The terms "% molar equivalents" and "% mol. equiv.," as used herein, refer to molar composition of salts expressed as a percent of GHB (or "oxybate") equivalents. For example, formulations and/or pharmaceutical compositions as described herein comprise mixtures with varying percentages of oxybate, expressed as % molar equivalents (% mol. equiv.) of Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. Those skilled in the art will understand that as each GHB unit is considered to be one molar equivalent, the monovalent cations, Na^+ and K^+, have one molar equivalent per salt, and the divalent cations, Mg^{+2} and Ca^{+2}, have two molar equivalents per salt. A sample calculation of % mol. equiv. is provided in the Examples of the present disclosure. For convenience in comparing various salt compositions at the same oxybate molar dose, compositions expressed as percentages in this application refer to molar equivalent percentage (% molar equivalents) of each oxybate salt. This is usually close to, but not the same as, the composition that would be expressed as wt/wt %.

The term, "buffering agent," as used herein, refers to a weak acid or base used to maintain the pH of a solution near a chosen pH value after the addition of another acidic or basic compound. The function of such an agent is to prevent the change in pH when acids or bases are added to a solution. Such agents may be acids, bases, or combinations thereof.

The term, "adjusting agent," as used herein, refers to an acid or base used to alter the pH of a solution to a chosen pH value. The function of such an agent is to alter the pH of a solution to the desired value subsequent to the addition of acidic or basic compounds.

The term, "acid," as used herein, refers to a substance which accepts a share in a pair of electrons. Such substances include malic acid, citric acid, acetic acid, boric acid, lactic acid, hydrochloric acid, phosphoric acid, sulfuric acid, sulfonic acid, nitric acid, and the like.

The term, "base," as used herein, refers to a substance which shares a pair of electrons. Such substances include sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, and the like.

The term, "chemically stable," as used herein, refers to a chemical compound which is not particularly reactive in a specific environment and retains its useful properties on a timescale of its expected usefulness. Specifically, the usefulness of the compound is maintained in the presence of air, moisture, or heat. Conversely, the compound lacks chemical stability if it decomposes under the conditions of a specific environment. As used herein in certain embodiments, "chemically stable" may mean resistant to degradation of GHB into its known or unknown decomposition elements. The level of GBL that is acceptable can be up to 0.15% of the formulation as per the ICH guidelines for shelf-life determination.

The term, "microbial," as used herein, refers to a microscopic organism that comprises either a single cell, cell cluster or multicellular organism.

The term "resistant to microbial growth" or "resistant to microbial challenge," as used herein, means that the compositions or formulations meet the criteria set by the Food and Drug Administration and the U.S. Pharmacopoeia for products made with aqueous bases or vehicles, which for bacteria means not less than a 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days, and for yeast and molds, no increase from the initial calculated count at 14 and 28 days.

The term, "preservative," as used herein, refers to a naturally occurring or synthetically produced substance which can be added to food, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by chemical decomposition.

The term, "formulation," as used herein, refers to a stable and pharmaceutically acceptable preparation of a pharmaceutical composition disclosed herein.

The term, "liquid formulation," as used herein, refers to a water-based formulation, in particular, a formulation that is an aqueous solution.

The term, "low volume" or "low aqueous volume" or "reduced volume," as used herein, refers to an aqueous solution of about 100 mL or less.

The term, "volume of administration" as used here, refers to the volume of aqueous material used to ingest or swallow the formulations and/or pharmaceutical compositions comprising the GHB salts, as disclosed herein, including before or immediately after the formulations and/or pharmaceutical compositions are ingested or swallowed. This amount can, for example, include the formulations and/or pharmaceutical disclosed herein and any additional aqueous material used to dilute, wash down or chase the formulations and/or pharmaceutical compositions. The additional aqueous material includes for example, water and flavored beverages.

The term, "eating" as used herein, refers to ingesting or consuming calories and/or nutrients by way of solid or liquid food substances.

The term, "cataplexy," as used herein, refers to a condition where a patient exhibits a sudden and transient loss of muscle tone, often triggered by emotions.

The term, "daytime sleepiness," as used herein, refers to a condition where a patient exhibits persistent sleepiness, and often a general lack of energy, even after apparent adequate night time sleep.

The term, "narcolepsy," as used herein, refers to a chronic sleep disorder characterized by excessive sleepiness and sleep attacks at inappropriate times.

The term, "apnea," as used herein, refers to a condition where a patient suspends external breathing.

The term, "sleep time disturbances," as used herein, refers to a condition where a patient exhibits abnormal sleep patterns. Sleep time disturbances can be serious enough to interfere with normal physical, mental and emotional functioning.

The term, "sleep paralysis," as used herein, refers to a condition in which a patient who is falling asleep or awakening form sleep experience an inability to move. It is a transition state between wakefulness and rest characterized by complete muscle weakness.

The term, "hypnagogic hallucination," as used herein, refers to a transition state between wakefulness and sleep where a patient experiences vivid hallucinations.

The term, "sleep arousal," as used herein, refers to a condition where a patient engages in sexual acts while still asleep.

The term, "insomnia," as used herein, refers to a condition where a patient has difficulties falling asleep and maintaining sleep.

The term, "nocturnal myoclonus," as used herein, refers to a condition where a patient has repetitive movement of the limbs during sleep or even wakefulness which is sometimes confused with a seizure.

The term "flavoring" or "flavoring agent," as used herein, refers to a substance that alters the flavor of the composition during oral consumption. A type of "flavoring agent" would be a sweetener.

The term "coloring" or "coloring agent," as used herein, refers to a substance that alters the color of the composition.

The term "bioequivalent", as used herein, describes a formulation and/or pharmaceutical composition that is therapeutically equivalent to a reference product (e.g. Xyrem®) when given under the same conditions in a pharmacokinetic evaluation conforming to FDA Guidance on Bioequivalence Testing; regardless of biopharmaceutical class. A value that is "bioequivalent", as used herein, is meant to refer to a pharmacokinetic value (such as the Cmax or AUC of a formulation described herein) that exhibits substantially similar pharmacokinetic profiles or therapeutic effects. Bioequivalence may be demonstrated through several in vivo and in vitro methods. These methods may include, for example, pharmacokinetic, pharmacodynamic, clinical and in vitro studies. In some embodiments, bioequivalence may be demonstrated using any suitable pharmacokinetic measures or combination of pharmacokinetic measures known in the art, including loading dose, steady-state dose, initial or steady-state concentration of drug, biological half-life, elimination rate, area under the curve (AUC), clearance, the peak blood or plasma concentration (Cmax), time to peak concentration (Tmax), bioavailability and potency. In some embodiments, a value is bioequivalent to a reference pharmacokinetic value when the geometric mean of the AUC and/or the Cmax is between 80% and 125% (e.g., at 90% confidence interval) of the reference pharmacokinetic value.

In some embodiments, a pharmaceutical composition is bioequivalent to a reference pharmaceutical composition when the pharmaceutical composition produces an average Cmax and/or AUC that is substantially the same as the Cmax and/or AUC of the reference pharmaceutical composition when administered under the same conditions. In some embodiments, a pharmaceutical composition is bioequivalent to a reference pharmaceutical composition when the pharmaceutical composition produces a Cmax and/or AUC that is within 80% and 125% of the Cmax and/or AUC of the reference pharmaceutical composition when administered under the same condition. For example, a pharmaceutical composition is bioequivalent to Xyrem® when the pharmaceutical composition produces an average Cmax and/AUC is between 80% and 125% of the Cmax and/or AUC of Xyrem® when administered under the same conditions.

The expression "consists essentially of" as used herein, means that specific further components can be present in a mixture or composition, namely those not materially affecting the essential characteristics of the mixture or composition.

6.2 Pharmaceutical Compositions Comprising Salt Mixtures of GHB

In certain aspects, provided herein are pharmaceutical compositions comprising gamma-hydroxybutyrate (GHB) and one or more pharmaceutically acceptable cations of an alkali metal or an alkaline earth metal. As used herein, "alkali metal" means any of the elements found in Group IA of the periodic table, including, for example, lithium, sodium, and potassium. As used herein, "alkaline earth metal" means any of the elements found in Group II of the periodic table, including, for example, magnesium and calcium.

In certain embodiments, the pharmaceutical compositions comprise GHB and more than one pharmaceutically acceptable cations of an alkali metal or an alkaline earth metal.

In certain embodiments, the pharmaceutical compositions comprise GHB and more than one (two or more) cations selected from the group consisting of Na^+, K^+, Mg^{+2}, and Ca^{+2}. In certain embodiments, the pharmaceutical compositions comprise GHB and all three cations selected from the group consisting of Na^+, K^+, and Ca^{+2}. In certain embodiments, the pharmaceutical compositions comprise less than 100% of the cation Na^+, so as to minimize the amount of sodium, particularly in patients with hypertension, heart disease, renal disease or at risk of stroke or to improve the taste of the compositions. In certain embodiments, the pharmaceutical compositions comprise from about 50% to

about 80% of the cation Na^+. In other embodiments, the pharmaceutical compositions comprise from about 0% to about 40% of the cation Na^+. Each embodiment has a different advantage.

In certain aspects, provided herein are pharmaceutical compositions comprising salts of GHB. As used herein, the term "salt of GHB" or "salts of GHB" is used interchangeably with the term "cation." For example, a pharmaceutical composition comprising GHB and the four cations Na^+, K^+, Mg^{+2}, and Ca^{+2} will be understood by those skilled in the art to also mean a pharmaceutical composition comprising the salts Na.GHB, K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. It will be also understood by those skilled in the art that such salts may be in solid form, or may be in partially or fully solvated form, for example, as when dissolved in an aqueous medium. It will be further understood by those skilled in the art, that, depending on the solubility of the salt in the aqueous medium, that the salt may be present in the aqueous medium as solvated cation(s) and anion(s), or as a precipitated solid.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises Na.GHB, and further comprises any one of the salts selected from the group consisting of K.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, K.GHB, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB, $Mg.(GHB)_2$, and $Ca.(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises Na.GHB and K.GHB. In certain embodiments, the pharmaceutical composition comprises Na.GHB, K.GHB, and $Mg.(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises Na.GHB and $Mg.(GHB)_2$.

The amounts of the cations below are described in various ranges. The cations can be present in the ranges found in U.S. Pat. Nos. 8,591,922; 8,901,173; and 9,132,107.

In certain embodiments, the Na.GHB salt is present in the mixture in a percentage of at least 50%. In certain embodiments, the Na.GHB salt is present in about 50% to about 80%. In certain embodiments, the Na.GHB salt is present in about 50% to about 70%. In certain embodiments, the Na.GHB salt is present in about 50% to about 60%. In certain embodiments, the Na.GHB salt is present in about 50% to about 55%. In certain embodiments, the Na.GHB salt is present between 40% and 50% and in others between 5% to 45%. In certain embodiments, the Na.GHB salt is present in about 5% to 35%. In certain embodiments, the Na.GHB salt is present in about 5% to 25%. In certain embodiments, the Na.GHB salt is present in about 5% to 10%.

In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 45% and 50% Na.GHB. In certain embodiments, the mixture comprises about 5% to 45% Na.GHB.

In certain embodiments, the mixture comprises at least 50% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 80% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 70% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 60% Na.GHB. In certain embodiments, the mixture comprises about 50% to about 55% Na.GHB. In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 5% to 45% Na.GHB. In certain embodiments, the mixture comprises about 5% to 35% Na.GHB. In certain embodiments, the mixture comprises about 5% to 25% Na.GHB. In certain embodiments, the mixture comprises about 5% to 10% Na.GHB.

In certain embodiments, the mixture comprises between 40% and 50% Na.GHB, and in others between 45% and 50% Na.GHB. In certain embodiments, the mixture comprises about 5% to 45% Na.GHB.

In certain embodiments, the remaining one, two or three or more cations that are present in the mixture in amounts to make up the remainder of the cations in the formulation and/or pharmaceutical composition. The amount of each depends on the amount of Na^+ and the amount of other cations. For example, if Na^+ is present at 50% and Ca^{+2} and K^+ are also present, then Ca^{+2} and K^+ can each be present in varying amount from 5-40% to add up to the remaining 50%. If Mg^{+2} is also present in the mixture then the non-sodium component 50% is divided three ways. In some embodiments, the mixture does not comprise a significant amount of $Mg.(GHB)_2$ or $Ca.(GHB)_2$, and therefore the formulation and/or pharmaceutical composition does not have a significant amount of $Mg.(GHB)_2$ or $Ca.(GHB)_2$. Care can be taken to adjust any specific cation concentration to levels that are acceptable to patients. It may not be preferred to add any cation to a level that might be disadvantageous to patients generally. For example, potassium has poor tolerability in solution at high doses given on an empty stomach and can also be a problem for patients with kidney impairment.

In certain embodiments, Na^+ is present at 50% and Ca^{2+} and K^+ are also present, then Ca^{2+} and K^+ can each be present in varying amount from 5-45% to add up to the remaining 50%.

In certain embodiments, the K.GHB, $Mg.(GHB)_2$ or $Ca.(GHB)_2$ salt is present in the mixture at about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the K.GHB, $Mg.(GHB)_2$ or the $Ca.(GHB)_2$ salt is absent.

In certain embodiments, the mixture comprises K.GHB, $Mg.(GHB)_2$ or the $Ca.(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% K.GHB. In certain embodiments, the mixture comprises about 0% $Mg.(GHB)_2$. In certain embodiments, the mixture comprises about 0% $Ca.(GHB)_2$.

In certain embodiments, the mixture comprises K.GHB in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%,

about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% K.GHB.

In certain embodiments, the mixture comprises Mg.$(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% Mg.$(GHB)_2$.

In certain embodiments, the mixture comprises Ca.$(GHB)_2$ in about 1% to about 5%, about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, about 30% to about 35%, or about 35% to about 40%, about 40% to about 45%, about 45% to about 50%, about 50% to about 55%, about 55% to about 60%, about 60% to about 65%, about 65% to about 70%, about 70% to about 75%, about 75% to about 80%, about 80% to about 85%, about 85% to about 90%, about 90% to about 95%, or about 95% to about 100%. In certain embodiments, the mixture comprises about 0% Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition has reduced sodium compared to Xyrem®, wherein the Na.GHB salt is present in the mixture at about 50% to about 80%.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises at least 50% of a sodium salt of Na.GHB, and further comprises one or more of the following salts, K.GHB, Ca.$(GHB)_2$ and Mg.$(GHB)_2$. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 80%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 70%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 60%. In certain embodiments, the Na.GHB salt is present in the mixture at about 50% to 55%.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more salts of GHB, wherein the mixture comprises of at least 50% of Na.GHB and further comprises one or more of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more salts of GHB, wherein the mixture consists essentially of at least 50% of Na.GHB and one or more of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of three or more salts of GHB.

In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the mixture does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the mixture consists of 50% to 80% Na.GHB, at least 10% K.GHB, and at least 10% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between 50% to 80% Na.GHB, between 30% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between 50% to 80% Na.GHB, between 10% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of between 50% to 80% Na.GHB, between 10% to 40% K.GHB, and between 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises about 50% to 80% Na.GHB, about 30% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises about 50% to 80% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of about 50% to 80% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between about 50% to 80% Na.GHB, between about 30% to 40% K.GHB, and between about 10% to 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between about 50% to 80% Na.GHB, between about 10% to 40% K.GHB, and between about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture consists essentially of between about 50% to 80% Na.GHB, between about 10% to 40% K.GHB, and between about 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between 50% and 60% Na.GHB, between 20% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between 50% and 60% Na.GHB, between 10% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises about 50% to about 60% Na.GHB, about 20% to about 40% K.GHB, and about 10% to about 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises about 50% to 60% Na.GHB, about 10% to 40% K.GHB, and about 10% to 20% Ca.$(GHB)_2$.

In certain embodiments, the composition comprises a mixture of three or more salts of GHB, wherein the mixture comprises between about 50% and about 60% Na.GHB, between about 20% and about 40% K.GHB, and between about 10% and about 20% Ca.$(GHB)_2$. In certain embodiments, the mixture comprises between about 50% and about 60% Na.GHB, between about 10% and about 40% K.GHB, and between about 10% and about 20% Ca.$(GHB)_2$.

In certain embodiments the mixture comprises 45% to 55% Na.GHB, 30% to 40% K.GHB, and 10% to 20% Ca.$(GHB)_2$. In certain embodiments the mixture comprises 48% to 52% Na.GHB, 32% to 36% K.GHB, and 14% to 18% Ca.$(GHB)_2$. In certain embodiments, the mixture does not have a substantial amount of Mg.$(GHB)_2$. In other embodiments, the mixture does not have a substantial amount of Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture of three GHB salts, wherein the mixture comprises at least 50% Na.GHB, and further comprises K.GHB and Ca.$(GHB)_2$, In certain embodiments, the mixture comprises between 50% and 60% of Na.GHB, between 10% and 40% K.GHB, and between 10% and 20% Ca.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$. In certain embodiments, the mixture does not comprise a substantial amount of Mg.$(GHB)_2$. In certain embodiments,

the Na.GHB, K.GHB, and Ca.$(GHB)_2$ salts are present in the mixture in a ratio of about 50%:34%:16%.

In certain embodiments, the pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture comprising between 40% and 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

In certain embodiments, the mixture comprises about 40% to about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the mixture comprises between about 40% and about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

In certain embodiments, the pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture essentially consists of about 40% to about 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg. $(GHB)_2$.

In certain embodiments, the pharmaceutical composition comprises a mixture which contains between 40% and 50% Na.GHB, wherein the composition is provided to the patient in an aqueous solution of between 25 and 100 mL. In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 40 and 75 mL. In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 55 and 65 mL.

In certain embodiments, the aqueous solution has a volume of about 25 mL to about 100 mL. In certain embodiments, the aqueous solution has a volume of about 40 mL to about 75 mL. In certain embodiments, the aqueous solution has a volume of about 55 mL to about 65 mL. In certain embodiments, the aqueous solution has a volume of about 60 mL.

In certain embodiments, the pharmaceutical composition comprises the mixture dissolved or dispersed in an aqueous solution of between 25 and 75 mL. In certain embodiments, the pharmaceutical composition comprises about 60 mL of an aqueous solution.

In certain embodiments, the pharmaceutical composition comprises between 25 and 100 mL of an aqueous solution. In certain embodiments the pharmaceutical composition comprises between 40 and 75 mL of an aqueous solution. In certain embodiments the pharmaceutical composition comprises between 55 and 65 mL of an aqueous solution.

In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 25 mL to about 100 mL. In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 40 mL to about 75 mL. In certain embodiments the pharmaceutical composition is an aqueous solution having a volume of about 55 mL to about 65 mL.

In certain embodiments, the pharmaceutical composition is bioequivalent to Xyrem® which is Na.GHB. In certain embodiments, the pharmaceutical composition produces an average maximum GHB plasma concentration (Cmax) that is substantially the same as the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition produces a Cmax that is within 80% and 125% of the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition produces an average maximum GHB plasma area under the curve (AUC) and Cmax that is substantially the same as Na.GHB. In certain embodiments, the pharmaceutical composition produces an AUC that is between 80% and 125% of the AUC of Na.GHB.

In certain embodiments, the pharmaceutical composition is bioequivalent to a pharmaceutical composition comprising about 100% Na.GHB when administered to a patient.

In certain embodiments, the average maximum GHB plasma concentration (Cmax) is within 10% of the Cmax of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, the AUC is within 10% of the AUC of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient.

In certain embodiments, the pharmaceutical composition is formulated as a liquid formulation, wherein the Na.GHB salt is present at less than 40%. In these embodiments, the pharmaceutical composition is more resistant to a food effect and has a lower Cmax compared to Na.GHB.

In certain embodiments, the pharmaceutical composition comprises a mixture of two or more GHB salts, wherein the mixture comprises less than 40% Na.GHB, and further comprises one or more of the following salts, K.GHB, Ca.$(GHB)_2$ and Mg.$(GHB)_2$. In certain embodiments, the Na.GHB salt is present in the mixture at about 0% to 30%. In certain embodiments, the Na.GHB salt is present in the mixture at about 5% to 25%. In certain embodiments, the Na.GHB salt is present in the mixture at about 5% to 10%.

In certain embodiments, the pharmaceutical composition comprises a mixture of three or more GHB salts, wherein the mixture comprises at least 10% K.GHB, at least 10% Ca.$(GHB)_2$ and at least 10% Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of two or three GHB salts, in addition to Na.GHB, wherein the mixture further comprises 20 to 80%, K.GHB, Ca.$(GHB)_2$ or Mg.$(GHB)_2$. In certain embodiments, the pharmaceutical composition comprises a mixture of three or more GHB salts, wherein the mixture comprises between 10 and 50% K.GHB, between 10 and 50% Ca.$(GHB)_2$ and between 10 and 50% Mg.$(GHB)_2$ for the non-sodium salts.

In certain embodiments, the Na.GHB, K.GHB, Mg.$(GHB)_2$, and Ca.$(GHB)_2$ salts are present in the mixture at a ratio of about 8%:23%:21%:48%, respectively.

6.2.1 Concentrations and pH Values

In certain embodiments, the pharmaceutical composition comprises an aqueous solution.

In certain embodiments, the concentration of the mixture of salts of GHB in the solution is about 250 mg/mL to about 750 mg/mL, about 350 mg/mL to about 650 mg/mL, about 400 mg/mL to about 600 mg/mL, about 450 mg/mL to about 550 mg/mL. In certain embodiments, the concentration of the mixture of salts of GHB in the solution is centered around 409 mg/mL GHB, which equates to 500 mg/mL Na.GHB. See U.S. Pat. No. 6,472,431, which is incorporated by reference in its entirety.

It will be understood that the maximum solubility of GHB is affected by the pH of the aqueous medium. For example, at about pH 4, the maximum amount of Na.GHB that can be dissolved is about 450 mg/mL. The value of pH that is conducive to GHB solubility increases so that the minimal pH that will dissolve 750 mg/mL GHB was found to be about pH 6.8.

Accordingly, in certain embodiments, the pharmaceutical composition has a pH of about 7.0 to about 9.0, about 7.0 to about 8.5, about 7.3 to about 8.5.

In certain embodiments, the pharmaceutical composition is chemically stable and resistant to microbial growth. In certain embodiments, the pharmaceutical composition is free of preservatives.

It will also be understood that the pH of the aqueous solution affects the resistance of the pharmaceutical composition to microbial growth at about 409 mg/mL GHB, which equates to, e.g., 500 mg/mL Na.GHB. For example, Na.GHB at this concentration (500 mg/mL) is resistant to microbial growth in an aqueous medium when the pH is between about pH 5 and pH 9. Compositions at about pH 6 to about pH 7.5 are particularly resistant to microbial growth. However, at concentrations of GHB greater than about 750 mg/mL above about pH 7.5, the resistance to microbial growth is reduced. See U.S. Pat. No. 6,472,431.

It will be further understood that the chemical stability of GHB is affected by pH. Accordingly, the method for preparing GHB, as described herein, particularly as disclosed in the specific examples, varies with pH. The impurity gamma butyrolactone (GBL) begins to form substantially if the pH is about 6 or less. Compositions with a pH of greater than about 6.0 are preferred to produce chemically stable formulations of GHB. Thus, a preferred range for chemically stable GHB would be from about pH 6 to about pH 9. However, any pH or range of pH values where a clinically acceptable amount of GBL is present is also contemplated as being preferred, and is encompassed by the present invention.

In certain embodiments, a pH adjusting or buffering agent may be added to the composition. The choice of a pH adjusting or buffering agent may affect the resistance to microbial challenge and/or the stability of GHB, as measured by the reduction in assayable GHB. Compositions of GHB, pH adjusted or buffered with malic or other acids are resistant to both microbial growth and chemical degradation of GHB, and are preferred. Other pH adjusting or buffering agents may be selected. Agents that adjust pH that are selected on this basis may undergo a taste testing study. However, any pH adjusting or buffering agent disclosed herein or as would be known to those skilled in the art is contemplated as being useful from the compositions or formulations disclosed herein. Of course, any salt, flavoring agent, excipient, or other pharmaceutically acceptable addition described herein, or as would be known to those skilled in the art, is contemplated as being useful for the compositions or formulations disclosed herein. See U.S. Pat. No. 6,472,431, and Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013, each of which is hereby incorporated by reference in its entirety.

In certain embodiments, the pH adjusting or buffering agent is an acid. In certain embodiments, the pH adjusting or buffering agent is an inorganic acid or an organic acid. In certain embodiments, the pH adjusting or buffering agent is selected from the group consisting of malic acid, citric acid, acetic acid, boric acid, lactic acid, hydrochloric acid, phosphoric acid, sulfuric acid, sulfonic acid, and nitric acid. In certain embodiments, the pH adjusting or buffering agent is malic acid. See U.S. Pat. No. 6,472,431.

6.2.2 Formulations

The aqueous solutions disclosed herein typically comprise an effective amount of GHB, or a salt or mixture of salts of GHB as disclosed herein, which may be dissolved or dispersed in a pharmaceutically acceptable carrier and/or an aqueous medium.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is not appropriate. Supplementary compatible active ingredients can be incorporated into the compositions. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the Food and Drug Administration (FDA). See Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013.

In certain embodiments, the compositions disclosed herein are provided in a formulation, preferably, a liquid formulation, although solid formulations are also contemplated. For any examples of excipients, colorants, flavorants, or other components of the formulation; see Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013.

In certain embodiments, the formulation is chemically stable and resistant to microbial growth. In certain embodiments, the formulation does not need, and may be free of preservatives. In certain embodiments, the level of gamma-butyrolactone (GBL) is 0.1% or less of the formulation. However, if preservatives are added they may include, but are not limited to, xylitol, sodium benzoate, methylparaben, propyl gallate BP, sorbic acid, chlorobutanol, dihydroacetic acid, monothioglycerol, potassium benzoate, propylparaben, benzoic acid, benzalkonium chloride, alcohol, benzoic acid, benzalkonium chloride, benzethonium chloride, benzyl alcohol, butylparaben, cetylpyridinium chloride, ethylenediamine, ethylparaben, ethyl vanillin, glycerin, hypophosphorus acid, methylparaben, phenol, phenylethyl alcohol, phenylmercuric nitrate, propylparaben, sassafras oil, sodium benzoate, sodium propionate, thimerosal and potassium sorbate. Preferred preservatives may be selected from the group comprising, but not limited to, xylitol, sodium benzoate, methylparaben, propylparaben and potassium sorbate. Xylitol is particularly preferred in certain compositions disclosed herein, because it acts as an preservative and a sweetener, is a caries preventative, is less laxative than other sweeteners, and is recommended for diabetics. See U.S. Pat. Nos. 8,324,275 and 8,952,062, and Remington, The Science and Practice of Pharmacy, 22nd Ed. 2013, each of which is incorporated hereby by reference in its entirety.

In certain embodiments, the formulation is suitable for oral administration.

In certain embodiments, the formulation additionally comprises a flavoring agent. Preferred sweeteners or flavoring agents would be microbially non-metabolizable. Especially preferred sweeteners or flavoring agents would be carbohydrates such as xylitol and sorbitol. Such flavoring agents include, but are not limited to, acacia syrup, anethole, anise oil, aromatic elixir, benzaldehyde, benzaldehyde elixir-compound, caraway, caraway oil, cardamom oil, cardamom seed, cardamom spirit, cardamom tincture-compound, cherry juice, cherry syrup, cinnamon, cinnamon oil, cinnamon water, citric acid, citric acid syrup, clove oil, coca, coca syrup, coriander oil, dextrose, eriodictyon, eriodictyon fluidextract, eriodictyon syrup-aromatic, ethyl acetate, ethyl, vanillin, fennel oil, ginger, ginger fluidextract, ginger oleoresin, glucose, glycerin, glycyrrhiza, glycyrrhiza elixir, glycyrrhiza extract, glycyrrhiza extract-pure, glycyrrhiza fluidextract, glycyrrhiza syrup, honey, non-alcoholic elixir, lavender oil, citrus extract or oil, lemon oil, lemon tincture, mannitol, methyl salicylate, nutmeg oil, orange-bitter-elixir,

orange-bitter-oil, orange flower oil, orange flower water, orange oil, orange peel-bitter, orange-peel-sweet-tincture, orange spirit-compound, compound, orange syrup, peppermint, peppermint oil, peppermint spirit, peppermint water, phenylethyl alcohol, raspberry juice, raspberry syrup, rosemary oil, rose oil, rose water, saccharin, saccharin calcium, saccharin sodium, sarsaparilla syrup, sorbitol solution, spearmint, spearmint oil, sucralose, sucrose, syrup, thyme oil, tolu balsam, tolu balsam syrup, vanilla, vanilla tincture, vanillin or wild cherry syrup.

In certain embodiments, the formulation additionally comprises a coloring agent. Preferred coloring agents would be microbially non-metabolizable.

In certain embodiments, the formulation is administered in a single or multiple dosage regimen.

Any of the above formulations may be prepared and/or packaged as a powdered or dry form for mixing with an aqueous medium before oral administration, or they may be prepared in an aqueous medium and packaged. After mixing with an aqueous medium, preferably to prepare a solution, these formulations are resistant to both microbial growth and chemical conversion of GHB to GBL, thereby increasing the shelf-life of therapeutic formulations of GHB, or salt or mixture of salts of GHB, in an aqueous medium. These formulations then provide an easily titratable liquid medium for measuring the dosage of GHB, or salt or mixture of salts of GHB, to be administered to a patient. Additional embodiments of the composition and methods of preparation are described below and in the examples.

In certain embodiments, especially with Na.GHB amounts between 40% and 50%, the formulation is present in a low volume of aqueous solution. As described herein, by "low volume" it is meant to include an aqueous solution of about 100 mL or less, including the aqueous medium and any wash or chase volume, for administration of a single GHB dose. Preferably the low volume is between about 25 mL to 75 mL, or between 55 mL to 65 mL of total aqueous volume given to the patient. In certain embodiments, for example, formulations with reduced sodium, the formulation requires less aqueous volume in order to be ingested, is more palatable, provides better patient compliance, is more tolerable, and/or is bioequivalent in comparison to GHB formulations of Na.GHB. It should be understood by those skilled in these arts that 25-100 mL (or about 1-3 ounces) of fluid is an acceptable amount of aqueous solvent to dilute the formulations disclosed herein, in order to ingest, improve taste, and/or "wash down" the GHB salts. For certain individuals, having a reduced-volume for administration offers an improved nightly dosing regimen which may alleviate unwanted side-effects associated with consuming liquids before bedtime, such as bed-wetting, restlessness and/or other sleep time disturbances.

The GHB, or salt or mixture of salts of GHB disclosed herein, may be lyophilized for more ready formulation into a desired vehicle or medium where appropriate. The GHB or salt(s) thereof may also be formulated for parenteral administration, e.g., formulated for injection via intravenous, intraarterial, intramuscular, sub-cutaneous, intralesional, intraperitoneal or other parenteral routes. The preparation of a pharmaceutical composition that comprises an aqueous solution that contains GHB or salt(s) thereof as an active component or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including, e.g., aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions of the active compounds as free acid or pharmacologically acceptable salts can be prepared in water suitably mixed with hydroxypropyl cellulose and/or a pharmaceutically acceptable surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof as well as in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to further prevent the growth of microorganisms.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, or the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a substance, such as lecithin (e.g., a coating), by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by any of the preservatives described herein, or as would be known to those skilled in the art, including various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with, various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, or highly, concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent (although DMSO may not now be a permitted human drug) is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solu-

tions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of fluid or injected at the proposed site of infusion, (see, e.g., "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The GHB may be prepared in a formulation and/or pharmaceutical composition disclosed herein to comprise about 100 to about 10,000 milligrams per dose as administered to the patient. The typical dose range is approximately 4.5-9 g/day; see the Xyrem® Product Insert. Other dose ranges include 6-8 g/day multiple or single doses can be administered but it is typical to give two divided doses per day. The Xyrem® instructions recommend two equally divided doses.

In addition to the pharmaceutical compositions formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g., tablets or other solids; liposomal formulations; time release capsules, such as sustained or delayed release forms, including beads, pellets, or resins; and any other form currently used, including creams, which then may be admixed with an aqueous medium for oral administration.

One may also use nasal solutions or sprays, aerosols or inhalants in connection with the pharmaceutical compositions and/or formulations disclosed herein. Nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, the aqueous nasal solutions usually are isotonic and slightly buffered to maintain a pH of 5.5 to 6.5, though other pH ranges disclosed herein the specific examples, such as pH 3 to about pH 9, or pH 6 to about 7.5, are contemplated. In addition, preservatives, similar to those used in ophthalmic preparations, and appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known and include, for example, antibiotics and antihistamines and are used for asthma prophylaxis.

The preferred oral formulations may include such normally employed excipients, as, for example, pharmaceutical grades of xylitol, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders to be admixed with an aqueous medium. In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent or assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or the GHB or salt(s) thereof may be packaged separately from or in combination with the excipients, salts, flavorings or any other components described herein, to be admixed with an aqueous medium for oral or injectable formulations, or they may be incorporated directly with the food (i.e. a beverage) of the diet.

For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of tablets, buccal tablets or tabs, troches, capsules, elixirs, suspensions, syrups, wafers, and the like, to be admixed with an aqueous medium. Such compositions and preparations should contain at least 0.1% of the active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, natural as gum tragacanth, acacia, cornstarch, or gelatin or synthetic as polyvinyl acetate; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a natural or synthetic flavoring agent. When the dosage unit form is a capsule for admixing with a specific volume of an aqueous medium, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with sugar, natural or synthetic polymers, or both. A syrup or elixir may contain the active compounds, sucrose as a sweetening agent, a preservative, a dye and/or a flavoring.

One embodiment of the formulations disclosed herein can be a solid with different release properties. One embodiment is a unit dosage form that is a tablet for immediate release comprising a relatively high weight-percentage of sodium oxybate, in combination with a relatively small weight-percentage of total excipients. This permits the tablets to contain/deliver a pharmaceutically effective amount of sodium oxybate in each tablet with a delivery profile similar to that of the liquid form. The tablets are bioequivalent to the liquid form. See U.S. Pat. Nos. 8,771,735 and 8,778,398. Other embodiments provide controlled release dosage forms for delivery of GHB or salt(s) thereof. The controlled release dosage forms may incorporate both controlled release and immediate release formulations in a single unit dosage form. See U.S. Publication No. 2012/0076865. Another embodiment includes the use of both immediate release and controlled release forms mixed together or one after the other. In one embodiment the immediate release portion could be between 10-50%, or 20-30% and the controlled release portion comprising the remaining amount. In some embodiments the amounts of the different salts can be different in each of the immediate or controlled release portions.

Additionally, any excipient, salt, acid, pH-mediating, adjusting or buffering compound or agent, flavoring, solution, solvent, dispersion, glycerol, glycol, oil, antibacterial and antifungal agents, antibiotics and antihistamines, binders, disintegrating agents, lubricants, sweetening agents, or any other additive or ingredient from those enumerated above or in the examples, or in any pharmaceutically acceptable composition or carrier described herein, or as would be known by one of skill in the art, is contemplated for use in aqueous mediums or solid forms of the pharmaceutical compositions disclosed herein. One or more of these compositions may be packaged with GHB or salt(s) thereof, or packaged separately from GHB or salt(s) thereof prior to consumption. If packaged separately, useful pharmaceutical compositions may be obtained by mixing GHB or salt(s) thereof with the other components with an aqueous medium prior to consumption. Such components may be packaged in a kit, described below.

Also provided herein are therapeutic kits comprising GHB, or a salt or mixture of salts of GHB, as disclosed herein. Such kits will generally contain, in suitable container, a pharmaceutically acceptable formulation of the GHB or salt(s) thereof. The kit may have a single container, or it may have distinct container for each component, or distinct container for various combinations of components.

When the components of the kit are provided in one or more liquid formulations, the liquid formulation is an aqueous medium, with a sterile aqueous solution being particularly preferred. The pharmaceutical compositions may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, vial, ampule or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, or even applied to and mixed with the other components of the kit.

However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, pouch syringe or other container means, into which the formulation or components thereof are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

The kits will also typically include a means for containing the vials in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained.

In certain embodiments, the kits contain one or more bottles of liquid formulation comprising GHB or salt(s) thereof, two dosing cups with child-resistant caps, a liquid measuring device and a medication guide.

In certain embodiments, the kits contain two different GHB formulations in separate bottles. In certain embodiments, the kits contain two bottles of liquid formulation comprising GHB or salt(s) thereof, wherein two different formulations are provided in at least two separate bottles. In certain embodiments, the kits contain two or more bottles of liquid formulation comprising GHB or salt(s) thereof, wherein two different formulations are provided in at least two separate bottles, and wherein also provided are two dosing cups with child-resistant caps, one or more liquid measuring device and a medication guide. Preferably, the two different formulations are a first-dose formulation comprising an aqueous solution, the aqueous solution is a mixture of two or more GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and the second-dose formulation comprising an aqueous solution comprising from 50% to about 80% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$.

Irrespective of the number or type of containers, the kits may also comprise, or be packaged with, an instrument for assisting with the injection/administration or placement of the pharmaceutical composition within the body of an animal. Such an instrument may be a drinking cup, syringe, pipette, or any such medically approved delivery vehicle. Where two more formulations are provided in the kit, optionally, one or more of the instruments or formulations can be color-matched or labeled to indicate which of the two doses are contained within it. Furthermore, the drug product containers can be differentiated by color, shape or other identifying features. The containers can be bound together (for example, by shrink wrapping) or assembled into the kit in such a way to minimize misplacement or discourage dispensing of one product for both dosings. Where two or more formulations are provided as granules or other rapidly dissolving dosage form, twin sachets with a perforated divider can facilitate dose preparation. These could be labeled, for example, as "1st dose" and "2nd dose".

Furthermore and to distinguish between prepared formulations prior to administration, one or both of the formulations can include a flavorant, odorant, or colorant to render it substantially different from the other. The additive may also be provided separately in the kit so that it can be added to the water either immediately before or after dispensing each formulation. Also, the administration devices for each dose may be distinguished based on a number of features such as color, shape, etc. so that that patient can easily administer each dose.

6.2.3 Methods of Treatment

All the pharmaceutical compositions and formulations provided herein can be used in all the methods provided herein. For example, the pharmaceutical compositions and formulations provided herein can be used in all the methods for treating all diseases, disorders or conditions provided herein. Thus, the pharmaceutical compositions and formulations provided herein are for use as a medicament. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy. In certain embodiments, the pharmaceutical compositions and formulations provided herein are for use in a method for treating a disease or condition in a subject that is suitable to treatment by GHB, comprising administering a pharmaceutical composition or formulation disclosed herein.

The pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, are also contemplated to be useful in the treatment of any of these disorders or conditions in patients. GHB has also been used alone as a narcotic in patients with terminal cancer. GHB has been used with other analgesics, neuroleptics, or with a subliminal barbiturate dose for use as an anesthesia. It is also contemplated that the pharmaceutical compositions and formulations disclosed herein may be used as a narcotic, hypnotic, or as a soporific. It is further contemplated that the pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, may be used in combination with analgesics, neuroleptics or barbiturates for use as an anesthesia. See the methods described at the end of U.S. Pat. No. 6,472,431.

The pharmaceutical compositions and formulations comprising mixed salts of GHB, disclosed herein, may be prepared and administered by any of the means described herein, particularly those described in the section "Formulations" and the examples, or by any means as would be known to those of skill in the art.

Accordingly, in certain aspects, are methods of treatment comprising administration to a patient of the pharmaceutical compositions or formulations comprising mixed salts GHB disclosed herein.

In certain embodiments, the pharmaceutical compositions or formulations comprising mixed salts of GHB, disclosed herein, are useful in the treatment of cataplexy or daytime sleepiness in a patient who has been diagnosed with narcolepsy.

In certain embodiments, the pharmaceutical compositions or formulations comprising mixed salts of GHB, disclosed herein, are useful in the treatment of conditions responsive to GHB, for example, sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

Accordingly, in certain embodiments, provided herein is a method for treating a disease or condition in a subject that is suitable to treatment by GHB, comprising administering a pharmaceutical composition or formulation disclosed herein.

In certain embodiments, also provided herein is a method of treating a disease or condition that is suitable for treatment with GHB wherein the method comprises administering to a patient a pharmaceutical composition comprising from 50% to about 80% of Na.GHB, wherein the pharmaceutical composition is in an oral dosage form and wherein administration of the pharmaceutical composition produces a GHB Cmax which is bioequivalent to the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In preferred embodiments, the disease is cataplexy and/or narcolepsy. In certain embodiments, the disease or condition is selected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In certain embodiments, the mixture of salts which from about 50% to about 80% of Na.GHB further comprises one or more salts selected from the group consisting of K.GHB and Ca.$(GHB)_2$.

In certain embodiments, also provided herein is a method of treating a disease or condition that is suitable for treatment with GHB wherein the method comprises administering to a patient a pharmaceutical composition of GHB comprising less than 100 mL of an aqueous solution, wherein the aqueous solution comprises a mixture of two or more salts of GHB, the mixture comprising between 40% and 50% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the disease is cataplexy and/or narcolepsy.

In certain embodiments, when administered to a patient, the pharmaceutical composition produces a GHB Cmax which is within 10% of the Cmax of Na.GHB. In certain embodiments, the Cmax is within 10% of the Cmax of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, when administered to a patient, the pharmaceutical composition produces a GHB Cmax that is bioequivalent to the Cmax of Na.GHB. In certain embodiments, the pharmaceutical composition is bioequivalent to a pharmaceutical composition comprising about 100% Na.GHB when administered to a patient. In certain embodiments, the AUC is within 10% of the AUC of a pharmaceutical composition comprising about the same amount of 100% Na.GHB when administered to a patient. In certain embodiments, the pharmaceutical composition does not comprise a substantial amount of Mg.$(GHB)_2$ or Ca.$(GHB)_2$. In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure. In preferred embodiments, the disease is cataplexy and/or narcolepsy. In certain embodiments, the disease or condition is selected from the group consisting of fibromyalgia and sleep disorders such as apnea, sleep time disturbances, narcolepsy, cataplexy, excessive daytime sleepiness (EDS), sleep paralysis, hypnagogic hallucination, sleep arousal, insomnia, and nocturnal myoclonus.

In certain embodiments, the methods of treatment comprising administration of the pharmaceutical compositions or formulations comprising mixed salts GHB disclosed herein.

In certain embodiments, the method comprises oral administration of the pharmaceutical compositions or formulations comprising mixed salts GHB, disclosed herein, in a multiple dosage regimen.

In certain embodiments, the multiple dosage regimen comprises one or more steps, as follows: (i) diluting an aqueous solution comprising about 409 mg/mL of gamma-hydroxybutyrate (GHB) with an aqueous medium to provide a first dose of the mixture of salts; (ii) diluting an aqueous solution comprising about 409 mg/mL of GHB with an aqueous medium to provide a second dose of the mixture of salts; (iii) orally administering to a patient having narcolepsy the first dose; and (iv) orally administering to the patient having narcolepsy the second dose within 2.5 to 4 hours following the first dose. The first and/or second doses can be administered according to the instructions on the label as appropriate.

In certain embodiments, two nightly doses of GHB or a salt there are administered to the patient.

In certain embodiments, the first dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution of a mixture of two or more GHB salts, the mixture comprising less than 40% Na.GHB, and further comprising one, two, three or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the first dose is administered within 4 hours of eating and produces a GHB Cmax which is less than the Cmax of Na.GHB; and the second dose of GHB salts is a pharmaceutical composition of GHB comprising a mixture of two or more GHB salts, the mixture comprising at least 50% of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the second dose produces a GHB Cmax which is substantially equivalent to the Cmax of Na.GHB. In certain embodiments, the multiple dosage regimen comprises one or more steps, as follows: (i) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising 0% to 40% Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, with an aqueous medium to provide a first dose of GHB salts; (ii) diluting an aqueous solution comprising a mixture of two or more GHB salts, the mixture comprising from about 50% to about 80%

of Na.GHB, and further comprising one or more salts selected from K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, to provide a second dose of GHB salts; (iii) orally administering the first dose to a patient suitable for treatment with GHB; and (iv) orally administering the second dose to the patient within 2.5 to 4 hours following the first dose. In preferred embodiments, the patient is suitable for treatment with GHB has cataplexy or narcolepsy.

In certain embodiments, the first dose comprises a pharmaceutical composition comprising less than 40% Na.GHB and at least two other GHB salts selected from the group of K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$. In certain embodiments, the first dose is administered within 4 hours of eating. In certain embodiments, the mixture further comprises two or more salts selected from the group consisting of K.GHB, Ca.$(GHB)_2$, and Mg. $(GHB)_2$.

In certain embodiments, the disease or condition is selected from the group consisting of a sleeping disorder, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, a neurological disorder, an endocrine disturbance, hypoxia or anoxia of tissues, and an increased level of intracranial pressure.

In certain embodiments, the first dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution of less than 100 mL, the aqueous solution comprises a mixture of three GHB salts, the mixture comprising less than 50% Na.GHB, and further comprising one or more salts selected from between 10-60% K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein the first dose is administered within 4 hours of eating and produces a GHB Cmax which is less than the Cmax of Na.GHB.

In certain embodiments, the second dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution, the aqueous solution comprising from 50% to about 80% of Na.GHB, and from between 10-60% K.GHB, Ca.$(GHB)_2$, and Mg.$(GHB)_2$, and wherein administration of the second dose produces a GHB Cmax which is substantially bioequivalent to the Cmax of Na.GHB. In certain embodiments, the second dose of GHB salts is a pharmaceutical composition of GHB comprising an aqueous solution which comprises a mixture from 50% to about 80% of Na.GHB, and wherein administration of the second dose produces a GHB Cmax which is substantially bioequivalent to a composition comprising Na.GHB.

In certain embodiments, 4.5 and 9 grams/day are administered to the patient in two divided doses.

In certain embodiments, 6 and 8 grams/day are administered to the patient in two divided doses.

In certain embodiments, the disease or condition is selected from the group consisting of sleeping disorders, drug abuse, alcohol and opiate withdrawal, a reduced level of growth hormone, anxiety, analgesia, neurological disorders (e.g., Parkinson's Disease and depression), endocrine disturbances, hypoxia or anoxia of tissues (such as from stroke or myocardial infarction), or an increased level of intracranial pressure.

It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including: the metabolic stability and length of action, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

6.2.4 Methods of Making

In certain aspects, provided herein are some exemplary methods of making the compositions or formulations comprising mixed salts GHB disclosed herein. Several different methods of making have been reported in the literature (see, e.g., U.S. Pat. Nos. 4,393,236; 4,983,632; 6,472,431; 8,461,203; 8,591,922; 8,901,173; and 9,132,107; and U.S. Publication No. 2016/0058720, each of which is incorporated by reference in its entirety; see also Ferris and Went, 2012, *Forensic Science International* 216: 158-162). Those skilled in the art will recognize that these methods can be incorporated in the making of the compositions or formulations comprising mixed salts GHB disclosed herein. Other methods will be known to those of skill in the art.

In certain embodiments, mixtures of GHB salts can be made by direct reaction of GBL with an aqueous mixture of one of more of the following bases: sodium hydroxide, potassium hydroxide, calcium hydroxide, and magnesium hydroxide. After reaction the mixture may then be filtered under mild vacuum.

In certain embodiments, a solvent, such as water, is used to dissolve the GHB salt mixture to a desired concentration, for example, by adjusting the amount of water in the mixture.

In certain embodiments, the concentration of a GHB salt solution is adjusted by concentrating the mixture using standard methods, such as evaporators, reverse osmosis, and similar techniques known to those skilled in the art.

In certain embodiments, the method of making comprises reacting gamma-butyrolactone (GBL) with one or more bases selected from the group consisting of sodium hydroxide, potassium hydroxide, magnesium hydroxide, and calcium hydroxide.

In other embodiments, the method of making comprises, for example, reacting GBL with one or more of sodium carbonate, potassium carbonate, or magnesium carbonate to provide the sodium, potassium, and magnesium oxybate (Na.GHB, K.GHB, and Mg.$(GHB)_2$) mixture. Such embodiments are particularly suitable to avoid precipitation of calcium carbonate when carbonate salts of sodium, potassium, and/or magnesium are employed.

In still other embodiments, a solution of calcium oxybate can be transformed to a mixture of oxybate salts by exchanging with a mixture of cation exchange resins loaded with the desired cations. Alternatively, a solution of calcium oxybate can be transformed to a mixture of oxybate salts by precipitation with a mixture of acid salts of other cations when the calcium salt is practically insoluble. After filtration or other means of removing the precipitated calcium salt or the exchanged cation exchange resin, the mixed oxybate salt solution is obtained.

In other embodiments, a mixture of cations associated with oxybate may include a proton. This can be achieved in similar fashion as cation exchange or displacement precipitation described above, with the exception that a H-form cation exchange resin or the free acid or partially neutralized salt of the precipitating anion is employed, respectively. Ideally to promote chemical stability, such embodiments should be produced in solid form and suspended or dissolved in water upon administration. In yet another embodiment, the undissolved solid (exchanged cationic resin or precipitated salt) can be ingested with the dose provided neither dissolves appreciably in the GI tract.

In certain embodiments, the reaction is carried out in a single vessel. For example, a mixture of Na.GHB, K.GHB, Mg.$(GHB)_2$, and Ca.$(GHB)_2$ may be made by direct addition of GBL to in a single vessel containing an aqueous mixture of sodium hydroxide, potassium hydroxide, magnesium hydroxide, and calcium hydroxide.

In certain embodiments, the reaction is carried out in multiple vessels and the product is subsequently combined. For example, Ca.$(GHB)_2$ may be made by direct addition of GBL to aqueous sodium hydroxide, and the product combined with Mg.$(GHB)_2$.

In certain embodiments, the methods of making include methods of making the pharmaceutical compositions and formulations disclosed herein.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the and scope of the invention.

7. EXAMPLES

Example 1: Synthesis of Mixed Oxybate Solutions

The following synthetic examples provide exemplary syntheses of mixture of oxybate salts. Alternate methods of synthesizing mixtures of oxybate salts, including methods of synthesizing additional salts of oxybate are described below; still other alternate synthetic methods will be apparent to those skilled in the art. See also U.S. Pat. Nos. 8,461,203; 8,591,922; 8,901,173; and 9,132,107; and U.S. Publication No. 2016/0058720; each of which is incorporated by reference in its entirety.

Mixed oxybate salt solutions can be made conveniently by at least two methods. When multiple different formulations are desired, one of skill in the art can mix solutions of individual salts having the same molar oxybate concentration to arrive at the desired cation blend. On the other hand, for commercial implementation or single-batch manufacturing one can perform a one-pot reaction with GBL and the two or more bases in the desired cationic proportions. Both methods are described below.

Example calculations of molar equivalents and % wt/wt for salt mixtures are also shown below Table 1.

TABLE 1

Example Calculations

Base	Base MW	Purity	Grams Amount	Base mMols	Stoich. Ratio	Base mEQ	% molar equiv GHB	Salt	Salt MW	Salt mass grams	Salt wt/wt %	Conc mg/mL
NaOH	40.00	98.50%	1.398	34.43	1	34.43	8.5%	Na•GHB	126.09	4.34	8.5%	42.61
KOH	56.11	86.72%	7.337	113.40	1	113.40	28.0%	K•GHB	142.20	16.12	31.4%	158.29
$Ca(OH)_2$	74.10	99.00%	6.268	83.74	2	167.49	41.4%	$Ca•(GHB)_2$	246.27	20.62	40.2%	202.46
$Mg(OH)_2$	58.32	99.50%	2.611	44.55	2	89.09	22.0%	$Mg•(GHB)_2$	230.50	10.27	20.0%	100.80
Total			17.614	276.11		404.40	100.0%			51.36	100.0%	504.17

Base	Each of four bases used in this example
Base MW	Molecular weight of the base
Purity	Purity provided by manufacturer. It is assumed that impurities are non-reactive.
Gram Amount	Amount, in grams, of each base charged to the reaction
Base mMols	Corresponding amount, in millimoles, of pure base (that is, Purity × Gram-Amount × 1000/Base-MW)
Stoichiometry Ratio	The number of GHB moles reacted with each mole of base
Base mEQ	Base equivalents for reaction with GHB (that is, Base-mMols × Stiochiometry-Ratio). This is also the Oxybate or GHB equivalents value.
% molar equiv GHB	Molar composition of salts expressed as Percent of Oxybate Equivalents
Salt	The oxybate salt species
Salt MW	Molecular weight of the oxybate salt
Salt-mass-grams	Mass of salt produced by reaction (that is, Base-mMols × Salt-MW/1000)
Salt wt/wt %	Normalized weight percent
Conc. (mg/ml)	Concentration in mg/ml equivalent to a 3.97M Na-GHB solution (500 mg/ml sodium oxybate). That is, 3.97 × (% equiv-GHB) × (Salt-MW)/(Stoich. Ratio)

Example 1.1: Manufacturing Mixed Salts Solutions

Four individual oxybate salt solutions at equal oxybate strength (409 mg/mL) were made as follows:

Magnesium oxybate (Mg.$(GHB)_2$) solution was made by combining 124.6 g water and 20.36 g magnesium hydroxide in a magnetically-stirred 250 mL square glass bottle. 58.04 g of GBL was then added to the base suspension and then heated up to 80° C. with stirring. After 4 hours, a pH verification indicated completion of reaction (pH 8.5). Water was added to compensate for evaporation. The reaction mixture was then centrifuged, and supernatant filtered through 0.45µ. PVDF Stericup under vacuum. The pH of filtrate was 8.1. Yield: 177.4 g solution. Assay (HPLC-UV): 100.1%

Potassium oxybate (K.GHB) solution was made by adding 60.10 g potassium hydroxide to 144.01 g water in a magnetically-stirred 250 mL square glass bottle. After complete dissolution, 78.52 g GBL was weighed into a separate glass beaker. Approximately half the GBL was added initially with instant reaction, and then the solution was cooled in ice water to approximately 30° C. The remainder of the GBL was then added with stirring, and the solution maintained at 60° C. for 2.5 hours. The pH was 13.5. The pH was then adjusted to 8.1 by adding 10% HCl solution. Water was added to restore the initial reaction mass. The solution was then filtered through 0.45µ. PVDF Stericup under vacuum. Yield: 281.8 g solution. Assay (HPLC-UV): 98.6%.

Calcium oxybate (Ca.$(GHB)_2$) solution was made by combining 210.5 g water and 45.41 g calcium hydroxide in a magnetically stirred 500 mL square glass bottle. Next, 102.41 g GBL was added slowly while stirring, and then the reaction was maintained at 80° C. on a temperature-controlled hotplate (surface set point 183° C.). After 2 hours, the

mixture was cooled and water was added to compensate for evaporation. The solution was centrifuged, and supernatant was then filtered through 0.45μ. PVDF Stericup under vacuum. The initial pH of filtrate was 10.5, and was adjusted to 7.9 by addition of 10% HCl solution. Yield: 328.6 g solution. Assay (HPLC-UV): 99.0%

Sodium oxybate (Na.GHB) solution was made by adding 46.6 g sodium hydroxide to 200.1 g water in a magnetically stirred 500 mL square glass bottle. 99.00 g GBL was weighed into a separate beaker. After complete dissolution of the sodium hydroxide, about half of the GBL was added to the reaction mixture causing it to heat. After cooling to about 30° C. in ice water, the remaining GBL was added and then allowed to react with stirring on a hotplate at 60° C. for 2 hours. The pH after reaction was 12.36, and was adjusted to 8.13 by addition of 10% HCl solution. Water was added to restore the initial reaction mass. The solution was then filtered through a 0.45μ. PVDF Stericup under vacuum. Yield: 340.3 g. Assay (HPLC-UV): 100.6%.

For each desired oxybate salt mixture below, the individual solutions were blended volumetrically with an oral dosing syringe into a 250 mL glass beaker with stirring. The blend order, where applicable, was sodium, potassium, calcium, and then magnesium oxybate. 178 mg of sucralose was then added and dissolved. The target cation blends (in equivalents) and volumes of individual solutions used are shown in Table 2 below.

TABLE 2

Target Cation Blends and Volumes of Exemplary Solutions

	% equivalents				Volume (mL) of oxybate solution (#1-#4 above) for total batch 150 mL				Assay
Solution	Na	K	Ca	Mg	Na (#4)	K (#2)	Ca (#3)	Mg (#1)	% Label
507-A	33	34	33	0	49.5	51.0	49.5	0	98.9
507-G	23.3	19.2	40	17.5	35.0	28.8	60.0	26.3	99.2
507-C	33	0	48	19	49.5	0	72.0	28.5	100.0
507-D	50	34	16	0	75.0	51.0	24.0	0	98.8

Example 1.2: Direct, One-Pot Reaction Method to Achieve Various Mixtures

To achieve any combination of oxybate salts, the stoichiometry calculations are adjusted to reflect (a) the strength of individual bases and (b) the use of an excess for the weakest base (calcium or magnesium). The strength of bases used in the Example above were 99.7% (NaOH), 86.0% (KOH), 99.0% ($Ca.(OH)_2$), and 98.5% ($Mg.(OH)_2$). A 1% excess is applied as the weakest divalent base present (calcium or magnesium, in that order of precedence). A larger or smaller excess may be warranted, depending on the level of confidence in the assay values or repeatability of dispensing to the process. A larger excess will increase confidence in completing the reaction, but incur more filtration load. A smaller excess threatens to inadequately complete the reaction, resulting in higher than desired GBL levels.

To make 150 mL batches roughly equivalent in composition to those of Example 1.1, the stoichiometry is as shown in Table 3 below.

TABLE 3

Stoichiometry of Bases used for Exemplary Solutions

	grams base required				Excess		GBL	Water	Total
Solution	NaOH	KOH	$Ca(OH)_2$	$Mg(OH)_2$	As base	grams	grams	grams	grams
507-A	7.88	13.21	7.35	0.00	$Ca(OH)_2$	0.22	51.27	98.56	178.5
507-G	5.57	7.46	8.91	3.12	$Mg(OH)_2$	0.17	51.27	102.00	178.5
507-C	7.88	0.00	10.70	3.38	$Mg(OH)_2$	0.17	51.27	105.09	178.5
507-D	11.95	13.21	3.57	0.00	$Ca(OH)_2$	0.22	51.27	98.29	178.5

The water is weighed into a tared 250 mL beaker with spinbar. Next, bases are weighed and added in order of sodium, potassium, calcium, and magnesium as applicable. After sodium or potassium hydroxide is added, the mixture is stirred until complete dissolution is observed. The required excess is added at the same time as the respective base is charged. Next, 51.27 g of GBL is added slowly while monitoring temperature and with stirring. If the temperature exceeds about 80° C., then GBL addition is slowed until the temperature cools to about 60° C. After GBL addition is complete, the setup is moved to a 60° C. environmental chamber to complete the reaction. (Alternatively, a temperature-controlled hotplate can be employed.) Sodium and potassium hydroxide react almost instantly with GBL. $Ca.(OH)_2$ requires about 1 h to react at 60° C., and $Mg.(OH)_2$ requires about 3 h at 80° C. or overnight (12 h) at 60° C. Therefore, mixtures lacking $Mg.(OH)_2$ (507-A and 507-D) are held at 60° C. for about 1 h. Mixtures 507-G and 507-C are held at 60° C. overnight or 80° C. for 3 h.

After reaction, water is added to compensate for evaporation and restore the original reaction mass (178.5 g net). The reaction mixtures are then centrifuged followed by vacuum filtration through a 0.45μ. PVDF Stericup. Finally, the pH is adjusted with 10% HCl solution, as needed, to a value of 8.0. For mixtures containing magnesium, no adjustment is required if the pH is below 9. Finally, 0.18 g of sucralose is added and dissolved into the solution.

Example 2: Pharmacokinetic Testing of Formulations

This Example provides protocols and results for bioequivalence testing of the formulations disclosed herein. Four sets of bioequivalence testing were performed with various mixed salt formulations compared with Xyrem® as the reference. Unless stated otherwise, this and subsequent examples have oxybate salt concentrations stated in a "molar equivalent percent" basis. Furthermore, in the tables and figures where applicable:

"Treatment" refers to the formulation and the dosing regimen (fed or fasted), for which various formulations were tested at a dose equivalent to 4.5 g sodium oxybate.

"N" refers to the number of subjects for which evaluable results were obtained

"Vol" refers to the volume of administration (mL) given with the 9 mL dose of drug product

"Cmax" refers to the average of the maximum plasma concentration (in oxybate mg/L or ug/mL) achieved in individual patients

"Cmax Ratio" refers to the ratio of Cmax value compared to that of fasted state Xyrem® and expressed as a percentage

"AUC" refers to the area under the curve of plasma vs time, either the last time point where the concentration was above the limit of quantitation or projected out to infinite time and expressed in units of h*mg/L.

"AUC ratio" refers to the ratio of AUC to that of fasted state Xyrem® and expressed as percentage

"Na", "K", "Ca", and "Mg" refer to the cation content of the formulation given, in Molar Equivalent %, of sodium, potassium, calcium, and magnesium, respectively.

Example 2.1: Testing of Formulation "O"

Formulation "O" was manufactured as (equivalent %) 8% sodium, 23% potassium, 48% calcium, and 21% magnesium

oxybate at 409 mg/mL mixed salt concentration or 409 mg/mL oxybate. The four bases were suspended or dissolved in water, then gamma butyrolactone was added and the reaction mixture was held at 80° C. for about 3 hours. Subsequently, mixture was cooled and then depth filtered, carbon filtered, and then flowed through a polishing filter. Finally, sucralose was added to a level of 0.1% w/v in the final solution.

Formulation "O" was tested for bioequivalence relative to Xyrem® (Formulation "X", commercial sodium oxybate solution of the same molar concentration and comparable pH as "O") and in the fasted as well as fed state. The study was compliant with the FDA guidance for food effect studies ("Guidance for Industry: Food-Effect bioavailability and Fed Bioequivalence Studies", FDA December 2002), incorporated herein by reference in its entirety. In both fasted and fed treatments, the Guidance indicates that the drug product should be administered with 240 mL of water. Thirty-six patients were recruited and 34 patients completed successfully. The results are shown in FIG. **1** and in Table 4 below.

TABLE 4

Conditions and Results in Study 13-010 Using 240 mL Liquid Volume

Treatment	Number of Patients	Vol (mL)	Cmax (mg/L)	Cmax ratio	AUC (mg•h/L)	AUC ratio	% equivalent Na	K	Ca	Mg
O, fasted	34	240	102.3	76%	238.7	89%	8	23	48	21
O, fed	36	240	77.7	58%	216.0	81%	8	23	48	21
X, fasted	32	240	134.6	100%	268.1	100%	100	0	0	0
X, fed	36	240	84.9	63%	233.0	87%	100	0	0	0

Example 2.2: Testing of Blends of Xyrem® and Formulation "O"

As an extension to the study described in Example 2.1, the same formulation "O" and Xyrem® reference were tested in two different proportions to determine whether bioequivalence could be achieved with the same proportion of the three non-sodium cations but with higher sodium content. New patients were recruited for the single dose crossover study, but the study was otherwise done in a manner comparable to Example 2.1 except fewer patients were evaluated. The results are shown in FIG. **2** and Table 5 as expressed in mean values. Bioequivalence was not achieved even at 49% sodium (the confidence interval for that formulation was between 73.8-97.5%).

TABLE 5

Conditions and Results in Study 13-010 Part 2 using 240 mL Liquid Volume

Treatment	Number of Patients	Vol (mL)	Cmax (mg/L)	Cmax ratio	AUC (mg•h/L)	AUC ratio	% equivalent Na	K	Ca	Mg
2.5 g O + 2.0 g X, fasted	21	240	109.4	84%	241.3	96%	49	13	27	12
3.75 g O + 0.75 g X, fasted	19	240	98.18	75%	228.4	91%	23	19	40	18
X, fasted	17	240	130.2	100%	251.4	100%	100			

Example 2.3: Testing of Alternative Cationic Blends

To test for negative effects of certain cations and also to investigate other four-cation blends, the formulations of Example 1.1 were tested in a crossover fasted state bioequivalence study involving 35 patients. In contrast to the preceding two examples, the volume of administration was reduced to 60 mL. The results are shown in FIG. **3** and Table 6.

Surprisingly, as shown in FIG. **3** and Table 6, Formulation 507-D with 50% sodium met the bioequivalence criteria, as it had a Cmax ratio of 92% and nearly identical average plasma profile compared to Xyrem®. In contrast, Formulations 507-A and 507-C, both with 33% sodium but differing by exclusion of either potassium or magnesium, had nearly identical and lower Cmax values (78% and 76%, respectively), and therefore did not meet the bioequivalence criteria.

TABLE 6

Conditions and Resu ts in Study 15-008 using 60 mL Liquid Volume, n = 35 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	(mg•h/L)	ratio	Na	K	Ca	Mg
507-A, fasted (no Mg)	60	102.2	77%	241	85%	33	34	33	0
507-C, fasted (no K)	60	101.0	77%	252	89%	33	0	48	19
507-D, fasted (higher Na, No Mg)	60	120.8	92%	257	90%	50	34	16	0
507-G (3.75 g O + 0.75 g X, fasted	60	95.6	72%	246	87%	23	19	40	18
X, fasted	60	131.9	100%	284	100%	100	0	0	0

Example 2.4: Testing Effect of Dilution Volume

Formulation 507-D having 50% sodium and tested at 60 mL volume was bioequivalent to Xyrem®, yet the four-cation blend of Example 2.2 having 49% sodium and tested at 240 mL volume was not bioequivalent. The difference between the two results is statistically significant and meaningful. To determine whether or how the volume of administration affects behavior of formulations, Formulation "O" was tested and compared to Xyrem® in three treatments fasted with 60 mL volume given, fasted with 240 mL volume, and fed with 60 mL volume. Thus, six treatments were administered in a crossover fashion involving 33 patients in a food effect bioequivalence study. The results are shown in FIG. **4** and Table 7.

There is little difference in the primary PK parameters (Cmax and AUC) as a result of volume of administration; however, there appears to be a difference in the mean plasma profile for Xyrem® at the two volumes when given fasted (FIG. **4**).

TABLE 7

Results of Study JZP258-101, n = 33 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	Mg•h/L)	ratio	Na	K	Ca	Mg
O, fasted	60	93.0	77%	238	95%	8	23	48	21
O, fasted	240	92.7	74%	233	90%	8	23	48	21
O, fed	60	63.0	52%	202	80%	8	23	48	21
X, fasted	60	120.5	100%	251	100%	100	0	0	0

TABLE 7-continued

Results of Study JZP258-101, n = 33 patients

	Vol	Cmax	Cmax	AUC	AUC	% equivalent			
Treatment	(mL)	(mg/L)	ratio	Mg•h/L)	ratio	Na	K	Ca	Mg
X, fasted	240	125.9	100%	258	100%	100	0	0	0
X, fed	60	68.6	57%	206	82%	100	0	0	0

Although the effect of dilution volume on food effect was not directly challenged in a single study, comparison of data from two crossover studies is possible for formulations "O" and Xyrem®. Table 8 shows the comparison of data from study JZP258-101 for 60 mL dilution volume and from study 13-010 Part 1 for 240 mL dilution volume. The results indicate that formulation "O" has a reduced food effect compared to Xyrem® and that, in both cases, the higher dilution volume has a smaller food effect.

TABLE 8

Comparison of Food Effect at 60 mL and 240 mL dilution

Treatment	Cmax (mg/L)		AUC (mg · h/L)	
Volume	60 mL	240 mL	60 mL	240 mL
O, fasted	93.0	102.3	238	239
O, fed	63.0	77.7	202	216
Ratio of O, fed to O, fasted	68%	76%	85%	90%
X, fasted	120.5	134.6	251	268
X, fed	68.6	84.9	206	233
Ratio of X, fed to X, fasted	57%	63%	82%	87%

In similar fashion, comparison of fasted data across studies can be done. FIG. **5**A shows the Cmax ratio as a function of the percent of calcium in the formulation. FIG. **5**B shows the Cmax ratio as a function of the percent of sodium in the formulation. The calcium model was arrived at by stepwise regression of main effect and interaction of calcium % and volume of administration using JMP software (SAS Institute). Volume of administration and its interaction were both dropped as insignificant terms. (An alternative model process employing calcium % and diluted concentration—which is volume-dependent—provided no better fit.) The result has significant lack of fit.

On the other hand, when sodium level and sodium diluted concentration (and interaction) are considered, a significantly better fit to results was obtained. All three terms were significant at 90% confidence or better, yet the main effect of diluted sodium concentration was least significant of the

three). Sodium level and its interaction with diluted sodium concentration were highly significant, respectively). That model fit is shown in FIG. **5**B.

Example 3: Expected Pharmacokinetics of Two Formulations Dosed 4 Hours Apart

The following proposed test treatment consists of administering formulation "O" of preceding examples and administering a second dose of formulation "507-D" 4 hours later. The reference treatment consists of Xyrem® given in the same fashion. Test and reference treatments have the same oxybate dose and are administered in 60 mL of water in the evening approximately two hours after dinner. Plasma is sampled at the same intervals as in preceding examples.

The outcome can be estimated by assuming additive contributions from each dose based on the single dose PK evaluations presented in preceding examples. The expected results are shown in FIG. **6** compared to those of the reference Xyrem® given under the same conditions.

Example 4: Microbial Challenge

This Example demonstrates that a mixed oxybate salt having low sodium displays acceptable resistance to microbial growth. A solution having, on a molar equivalents basis, 8% sodium, 23% potassium, 48% calcium, and 21% magnesium oxybate salts (Na.GHB, K.GHB, Mg.$(GHB)_2$, and Ca.$(GHB)_2$) with a pH value of 8 and a total concentration of 409 mg/mL oxybate salts was tested for antimicrobial effectiveness according to USP<51>. Individual samples were inoculated with each of five microorganisms and stored for 28 days at 20-25° C. At 7, 14, and 28 days microbial enumeration tests revealed effective reductions for all strains, as shown in Table 9 below.

TABLE 9

Microbial Effective
Test of 8% Na•GHB, 23% K•GHB,
48% Ca•$(GHB)_2$, and 21%
Mg•$(GHB)_2$ at 409 mg/mL
Log reduction in colony forming units/mL

Organism	Day 7	Day 14	Day 28
S. aureaus	>5.2	>5.2	>5.2
E. coli	>5.7	>5.7	>5.7
P. aeruginosa	>5.8	>5.8	>5.8
C. albicans	3.0	>5.6	>5.6
A. niger	2.6	3.6	>4.2

What is claimed is:

1. A method of reducing food effect due to administration of gamma-hydroxybutyrate (GHB) in a patient having cataplexy in narcolepsy or excessive daytime sleepiness in narcolepsy, comprising:

orally administering to a patient in need thereof a pharmaceutically effective amount of a pharmaceutical composition of GHB in a unit dosage comprising at least one salt of GHB and a pharmaceutically acceptable carrier within four hours after eating;

wherein the pharmaceutical composition of GHB has reduced food effect as measured by C_{max} compared to an equal dose of immediate release liquid solution of Na.GHB, wherein the pharmaceutical composition comprises: about 5% to about 10% of Na.GHB; about 20% to about 25% of K.GHB; about 45% to about 50% of Ca.$(GHB)_2$; and about 20% to about 25% of Mg.$(GHB)_2$.

2. The method of claim **1**, wherein the composition is administered with food, immediately after eating, up to 30 minutes after eating, or up to two hours after eating.

3. The method of claim **1**, wherein the composition provides an AUC when administered within four hours after eating that is 80%-95% of the AUC when the composition is administered while fasting.

4. The method of claim **1**, wherein the composition provides an AUC when administered within four hours after eating that is 85%-90% of the AUC when the composition is administered while fasting.

5. The method of claim **1**, wherein the composition provides a C_{max} when administered within four hours after eating that is 55%-80% of the C_{max} when the composition is administered while fasting.

6. The method of claim **1**, wherein the composition provides a C_{max} when administered within four hours after eating that is 60%-75% of the C_{max} when the composition is administered while fasting.

7. The method of claim **1**, wherein the composition provides a C_{max} that is less than the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses at least four hours after eating.

8. The method of claim **1**, wherein the composition provides a C_{max} that is less than the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses within four hours after eating.

9. The method of claim **1**, wherein the composition provides a C_{max} that is less than 60% the C_{max} of an equal dose of immediate release liquid solution of Na.GHB administered in equally divided doses at least four hours after eating.

10. The method of claim **1**, wherein the composition provides a change in C_{max} when administered at least four hours after eating and within four hours after eating that is 10-60% less than the change in C_{max} of an equal dose of immediate release liquid solution of Na.GHB when administered in equally divided doses at least four hours after eating and within four hours after eating.

11. The method of claim **1**, wherein the pharmaceutical composition comprises 8% of Na.GHB; 23% of K.GHB; 48% of Ca.$(GHB)_2$; and 21% of Mg.$(GHB)_2$.

* * * * *

EXHIBIT 5

Guidance for Industry

Food-Effect Bioavailability and Fed Bioequivalence Studies

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

December 2002
BP

Guidance for Industry

Food-Effect Bioavailability and Fed Bioequivalence Studies

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Guidance For Industry[1]

Food-Effect Bioavailability and Fed Bioequivalence Studies

This guidance represents the Food and Drug Administration=s current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.

I. INTRODUCTION

This guidance provides recommendations to sponsors and/or applicants planning to conduct food-effect bioavailability (BA) and fed bioequivalence (BE) studies for orally administered drug products as part of investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), and supplements to these applications. This guidance applies to both immediate-release and modified-release drug products. The guidance addresses how to meet the BA and BE requirements in 21 CFR 320, 314.50 (d) (3), and 314.94 (a) (7) as they apply to oral dosage forms. This guidance provides recommendations for food-effect BA and fed BE study designs, data analysis, and product labeling. It also provides information on when food-effect BA and fed BE studies should be performed. [2]

II. BACKGROUND

Food effect BA studies are usually conducted for new drugs and drug products during the IND period to assess the effects of food on the rate and extent of absorption of a drug when the drug product is administered shortly after a meal (fed conditions), as compared to administration under fasting conditions. Fed BE studies, on the other hand, are conducted for ANDAs to demonstrate their bioequivalence to the reference listed drug (RLD) under fed conditions.

A. Potential Mechanisms of Food Effects on BA

[1] This guidance has been prepared by the Food Effect Working Group of the Biopharmaceutics Coordinating Committee in the Office of Pharmaceutical Science, Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA).

[2] See also the guidance for industry on *Bioavailablity and Bioequivalence Studies for Orally Administered Drug Products C General Considerations*.

Food can change the BA of a drug and can influence the BE between test and reference products. Food effects on BA can have clinically significant consequences. Food can alter BA by various means, including

- Delay gastric emptying
- Stimulate bile flow
- Change gastrointestinal (GI) pH
- Increase splanchnic blood flow
- Change luminal metabolism of a drug substance
- Physically or chemically interact with a dosage form or a drug substance

Food effects on BA are generally greatest when the drug product is administered shortly after a meal is ingested. The nutrient and caloric contents of the meal, the meal volume, and the meal temperature can cause physiological changes in the GI tract in a way that affects drug product transit time, luminal dissolution, drug permeability, and systemic availability. In general, meals that are high in total calories and fat content are more likely to affect the GI physiology and thereby result in a larger effect on the BA of a drug substance or drug product. We recommend use of high-calorie and high-fat meals during food-effect BA and fed BE studies.

B. Food Effects on Drug Products

Administration of a drug product with food may change the BA by affecting either the drug substance or the drug product. In practice, it is difficult to determine the exact mechanism by which food changes the BA of a drug product without performing specific mechanistic studies. Important food effects on BA are least likely to occur with many rapidly dissolving, immediate-release drug products containing highly soluble and highly permeable drug substances (BCS Class I) because absorption of the drug substances in Class I is usually pH- and site-independent and thus insensitive to differences in dissolution.[3] However, for some drugs in this class, food can influence BA when there is a high first-pass effect, extensive adsorption, complexation, or instability of the drug substance in the GI tract. In some cases, excipients or interactions between excipients and the food-induced changes in gut physiology can contribute to these food effects and influence the demonstration of BE. For rapidly dissolving formulations of BCS Class I drug substances, food can affect C_{max} and the time at which this occurs (T_{max}) by delaying gastric emptying and prolonging intestinal transit time. However, we expect the food effect on these measures to be similar for test and reference products in fed BE studies.

For other immediate-release drug products (BCS Class II, III, and IV) and for all modified-release drug products, food effects are most likely to result from a more complex combination of factors that influence the in vivo dissolution of the drug product and/or the absorption of the drug substance. In these cases, the relative direction and magnitude of food effects on formulation BA and the effects on the demonstration of BE are difficult, if not impossible, to predict without conducting a fed BE study.

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[3] See the guidance for industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*.

III. RECOMMENDATIONS FOR FOOD-EFFECT BA AND FED BE STUDIES

This section of the guidance provides recommendations on when food-effect BA studies should be conducted as part of INDs and NDAs and when fed BE studies should be conducted as part of ANDAs. For postapproval changes in an approved immediate- or modified-release drug product that requires in vivo redocumentation of BE under fasting conditions, fed BE studies are generally unnecessary.

A. Immediate-Release Drug Products

1. INDs/NDAs

We recommend that a food-effect BA study be conducted for all new chemical entities (NCEs) during the IND period.

Food-effect BA studies should be conducted early in the drug development process to guide and select formulations for further development. Food-effect BA information should be available to design clinical safety and efficacy studies and to provide information for the CLINICAL PHARMACOLOGY and/or DOSAGE AND ADMINISTRATION sections of product labels. If a sponsor makes changes in components, composition, and/or method of manufacture in the clinical trial formulation prior to approval, BE should be demonstrated between the to-be-marketed formulation and the clinical trial formulation.

Sponsors may wish to use relevant principles described in the guidance for industry on *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (SUPAC-IR guidance) to determine if in vivo BE studies are recommended. These BE studies, if indicated, should generally be conducted under fasting conditions.

2. ANDAs

In addition to a BE study under fasting conditions, we recommend a BE study under fed conditions for all orally administered immediate-release drug products, with the following exceptions:

- When both test product and RLD are rapidly dissolving, have similar dissolution profiles, and contain a drug substance with high solubility and high permeability (BCS Class I) (see footnote 3), or

- When the DOSAGE AND ADMINISTRATION section of the RLD label states that the product should be taken only on an empty stomach, or

- When the RLD label does not make any statements about the effect of food on absorption or administration.

B. Modified-Release Drug Products

We recommend that food-effect BA and fed BE studies be performed for all modified-release dosage forms.

1. INDs/NDAs

We recommend a study comparing the BA under fasting and fed conditions for all orally administered modified-release drug products.

When changes occur in components, composition, and/or method of manufacture between the to-be-marketed formulation and the primary clinical trial material, the sponsor may wish to use relevant principles described in the guidance for industry on *SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing, and Controls: In Vitro Dissolution Testing and In Vivo Bioequivalence Documentation* (SUPAC-MR guidance) to determine if documentation of in vivo BE is recommended. These BE studies, if indicated, should generally be conducted under fasting conditions.

2. ANDAs

In addition to a BE study under fasting conditions, a BE study under fed conditions should be conducted for all orally administered modified-release drug products.

IV. STUDY CONSIDERATIONS

This section provides general considerations for designing food effect BA and fed BE studies. A sponsor may propose alternative study designs and data analyses. The scientific rationale and justification for these study designs and analyses should be provided in the study protocol. Sponsors may choose to conduct additional studies for a better understanding of the drug product and to provide optimal labeling statements for dosage and administration (e.g. different meals and different times of drug intake in relation to meals). In studying modified-release dosage forms, consideration should be given to the possibility that co-administration with food can result in *dose dumping*, in which the complete dose may be more rapidly released from the dosage form than intended, creating a potential safety risk for the study subjects.

A. General Design

We recommend a randomized, balanced, single-dose, two-treatment (fed vs. fasting), two-period, two-sequence crossover design for studying the effects of food on the BA of either an immediate-release or a modified-release drug product. The formulation to be tested should be administered on an empty stomach (fasting condition) in one period and following a test meal

(fed condition) in the other period. We recommend a similar, two-treatment, two-period, two-sequence crossover design for a fed BE study except that the treatments should consist of both test and reference formulations administered following a test meal (fed condition). An adequate washout period should separate the two treatments in food-effect BA and fed BE studies.

B. Subject Selection

Both food-effect BA and fed BE studies can be carried out in healthy volunteers drawn from the general population. Studies in the patient population are also appropriate if safety concerns preclude the enrollment of healthy subjects. A sufficient number of subjects should complete the study to achieve adequate power for a statistical assessment of food effects on BA to claim an absence of food effects, or to claim BE in a fed BE study (see DATA ANALYSIS AND LABELING section). A minimum of 12 subjects should complete the food-effect BA and fed BE studies.

C. Dosage Strength

In general, the highest strength of a drug product intended to be marketed should be tested in food-effect BA and fed BE studies. In some cases, clinical safety concerns can prevent the use of the highest strength and warrant the use of lower strengths of the dosage form. For ANDAs, the same lot and strength used in the fasting BE study should be tested in the fed BE study. For products with multiple strengths in ANDAs, if a fed BE study has been performed on the highest strength, BE determination of one or more lower strengths can be waived based on dissolution profile comparisons (for details see the guidance on *Bioavailablity and Bioequivalence Studies for Orally Administered Drug Products - General Considerations.*

D. Test Meal

We recommend that food-effect BA and fed BE studies be conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. A high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) meal is recommended as a test meal for food-effect BA and fed BE studies. This test meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.[4] The caloric breakdown of the test meal should be provided in the study report. If the caloric breakdown of the meal is significantly different from the one described above, the sponsor should provide a scientific rationale for this difference. In NDAs, it is recognized that a sponsor can choose to conduct food-effect BA studies using meals with different combinations of fats, carbohydrates, and proteins for exploratory or label purposes. However, one of the meals for the food-effect BA studies should be the high-fat, high-calorie test meal described above.

[4] An example test meal would be two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes and eight ounces of whole milk. Substitutions in this test meal can be made as long as the meal provides a similar amount of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

E. Administration

Fasted Treatments: Following an overnight fast of at least 10 hours, subjects should be administered the drug product with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

Fed Treatments: Following an overnight fast of at least 10 hours, subjects should start the recommended meal 30 minutes prior to administration of the drug product. Study subjects should eat this meal in 30 minutes or less; however, the drug product should be administered 30 minutes after start of the meal. The drug product should be administered with 240 mL (8 fluid ounces) of water. No food should be allowed for at least 4 hours post-dose. Water can be allowed as desired except for one hour before and after drug administration. Subjects should receive standardized meals scheduled at the same time in each period of the study.

F. Sample Collection

For both fasted and fed treatment periods, timed samples in biological fluid, usually plasma, should be collected from the subjects to permit characterization of the complete shape of the plasma concentration-time profile for the parent drug. It may be advisable to measure other moieties in the plasma, such as active metabolites, and sponsors should refer to the guidance on *Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations* for recommendations on these issues. Consideration should be given to the possibility that co-administration of a dosage form with food can alter the time course of plasma drug concentrations so that fasted and fed treatments can have different sample collection times.

V. DATA ANALYSIS AND LABELING

Food-effect BA studies may be exploratory and descriptive, or a sponsor may want to use a food-effect BA study to make a label claim.[5] The following exposure measures and pharmacokinetic parameters should be obtained from the resulting concentration-time curves for the test and reference products in food-effect BA and fed BE studies:

- Total exposure, or area under the concentration-time curve ($AUC_{0\text{-inf}}$, $AUC_{0\text{-t}}$)
- Peak exposure (C_{max})
- Time to peak exposure (T_{max})
- Lag-time (t_{lag}) for modified-release products, if present
- Terminal elimination half-life
- Other relevant pharmacokinetic parameters

Individual subject measurements, as well as summary statistics (e.g., group averages, standard deviations, coefficients of variation) should be reported. An equivalence approach is

[5] Regulations on labeling requirements for a drug product submitted in an NDA can be found in 21 CFR part 201.

recommended for food-effect BA (to make a claim of no food effects) and fed BE studies, analyzing data using an average criterion. Log-transformation of exposure measurements (AUC and C_{max}) prior to analysis is recommended. The 90 percent CI for the ratio of population geometric means between test and reference products should be provided for $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and C_{max} (see guidance for industry on *Statistical Approaches to Establishing Bioequivalence*). For IND or NDA food-effect BA studies, the fasted treatment serves as the reference. For ANDA fed BE studies, the RLD administered under fed condition serves as the reference treatment.

The effect of food on the absorption and BA of a drug product should be described in the CLINICAL PHARMACOLOGY section of the labeling. In addition, the DOSAGE AND ADMINISTRATION section of the labeling should provide instructions for drug administration in relation to food based on clinical relevance (i.e., whether or not the changes in systemic exposure caused by co-administration with food results in safety or efficacy concerns, or when there is no important change in systemic exposure but there is a possibility that the drug substance causes GI irritation when taken without food).

For an NDA, an absence of food effect on BA is not established if the 90 percent CI for the ratio of population geometric means between fed and fasted treatments, based on log-transformed data, is not contained in the equivalence limits of 80-125 percent for either $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) or C_{max}. When the 90 percent CI fails to meet the limits of 80-125 percent, the sponsor should provide specific recommendations on the clinical significance of the food effect based on what is known from the total clinical database about dose-response (exposure-response) and/or pharmacokinetic-pharmacodynamic relationships of the drug under study. The clinical relevance of any difference in T_{max} and t_{lag} should also be indicated by the sponsor. The results of the food-effect BA study should be reported factually in the CLINICAL PHARMACOLOGY section of the labeling and should form the basis for making label recommendations (e.g., *take only on an empty stomach*) in the DOSAGE AND ADMINISTRATION section of the labeling. The following are examples of language for the package insert:

> A food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that the C_{max} and AUC were increased 57% and 45%, respectively, under fed conditions. This increase in exposure can be clinically significant, and therefore [the drug] should be taken only on an empty stomach (1 hour before or 2 hours after a meal)
>
> A food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that the C_{max} was decreased 15% while the AUC remained unchanged. This decrease in exposure is not clinically significant, and therefore [the drug] could be taken without regards to meals.

An absence of food effect on BA is indicated when the 90 percent CI for the ratio of population geometric means between fed and fasted treatments, based on log-transformed data, is contained in the equivalence limits of 80-125 percent for $AUC_{0\text{-}inf}$ ($AUC_{0\text{-}t}$ when appropriate) and C_{max}. In this case, a sponsor can make a specific claim in the CLINICAL PHARMACOLOGY or DOSAGE AND ADMINISTRATION section of the label that no food effect on BA is expected

provided that the T_{max} differences between the fasted and fed treatments are not clinically relevant. The following is an example of language for the package insert:

> The C_{max} and AUC data from a food-effect study involving administration of [the drug product] to healthy volunteers under fasting conditions and with a high-fat meal indicated that exposure to the drug is not affected by food. Therefore, [the drug product] may be taken without regard to meals.

For an ANDA, BE of a test product to the RLD product under fed conditions is concluded when the 90 percent CI for the ratio of population geometric means between the test and RLD product, based on log-transformed data, is contained in the BE limits of 80-125 percent for AUC and C_{max}. Although no criterion applies to T_{max}, the T_{max} values for the test and reference products are expected to be comparable based on clinical relevance. The conclusion of BE under fed conditions indicates that with regard to food, the language in the package insert of the test product can be the same as the reference product.

VI. OTHER CONSIDERATIONS

A. Sprinkles

In NDAs, the labeling of certain drug products (e.g., controlled-release capsules containing beads) can recommend that the product be sprinkled on soft foods, such as applesauce, and swallowed without chewing. For the labeling to indicate that the drug product can be sprinkled on soft foods, additional in vivo relative BA studies should be performed by sprinkling the product on the soft foods to be listed in the labeling (test treatment) and comparing it to the product administered in the intact form (reference treatment), then administering both on an empty stomach.

In ANDAs, BE of the test to the RLD is demonstrated in a single dose crossover study. Both treatments should be sprinkled on one of the soft foods mentioned in the labeling, usually applesauce. The BE data should be analyzed using average BE and the 90 percent CI criteria should be used to declare BE. If there are questions about other foods, the design, or the analysis of such BE studies, the sponsors and/or applicants should contact the Office of Generic Drugs.

B. Special Vehicles

For NDAs, the labeling for certain oral solution products (e.g., cyclosporine oral solution, modified) recommends that the solution be mixed with a beverage prior to administration. The BA of these products can change when mixed with different beverages due to the formation of complex mixtures and other physical-chemical and/or physiological factors. NDA sponsors should contact the Office of Clinical Pharmacology and Biopharmaceutics to determine what data should be submitted to support labeling.

In ANDAs, BE of the test to the RLD is demonstrated in a single-dose crossover study. Both treatments should be mixed with one of the beverages mentioned in the labeling. Sponsors

should provide evidence that BE differences would not be expected from the use of other listed vehicles. The BE data should be analyzed using average BE, and the 90 percent CI criteria should be used to declare BE. If there are questions about other vehicles, or the design or analysis of such BE studies, the sponsors and/or applicants should contact the Office of Generic Drugs.

EXHIBIT 5

FILED UNDER SEAL